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KEMENTERIAN KESIHATAN MALAYSIA

MOLECULAR PROFILING ASSAYS IN EARLY BREAST CANCER

MaHTAS

Malaysian Health Technology Assessment Section

Medical Development Division
Ministry of Health Malaysia

HEALTH TECHNOLOGY ASSESSMENT REPORT

MOLECULAR PROFILING ASSAYS IN EARLY BREAST CANCER

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Malaysian Health Technology Assessment Section (MaHTAS)
Medical Development Division
Ministry of Health Malaysia

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EXECUTIVE SUMMARY

BACKGROUND

Molecular profiling is a scientific approach that compare different types of samples (tissues, body fluid, etc) at a molecular level (DNA, mRNA or protein) on a global scale. The molecular profiling assay is a genomic technology for predicting individual patient's prognosis by interpreting the expression pattern of a panel of specific tumour-related genes. The transcription of specific set of genes is used as a surrogate marker for metastatic potential. The pattern of gene expression and the specific gene expression threshold levels are able to identify tumours with a more aggressive biology. Thereby it will quantify the risk of recurrence more accurately and the oncologist may plan for either less or more aggressive treatment. In early-stage breast cancer, the advances in molecular biology and pharmacology aids in better understanding of breast cancer and enables the design of effective therapy to target the cancer more efficiently. The molecular profiling assays aim to improve the use of chemotherapy in breast cancer by improving the categorisation of patients in accordance with risk and the identification of those patients who will gain most benefit from chemotherapy. There are several commercially available molecular profiling assays including Oncotype DX, Prosigna (Predictor Analysis of Microarray 50 [PAM 50]), EndoPredict and MammaPrint. This assessment was requested by a Senior Consultant Breast & Endocrine Surgery from Hospital Kuala Lumpur due to increasing demands from patients and clinicians to use gene assays profiling as part of the management of early breast cancer. However, in-depth knowledge of the different assays, their usefulness, and cost-effectiveness is not readily available for a sound decision making process by clinicians for the individual patient(s).

Technical features

The Oncotype DX, MammaPrint, Prosigna and EndoPredict tests are already approved by the Food and Drug Administration (FDA) agency and European Medicines Agency (EMA) for early-stage breast cancer to predict recurrence risk and guide adjuvant chemotherapy decisions. Oncotype DX is a 21-gene expression assay which was initially developed for women with lower grade, small tumours (<5cm) node-negative (N-), oestrogen receptor-positive (ER+) early-stage breast cancer. MammaPrint is a 70-gene assay which was previously generated for patients with lymph node-negative (LN-) early-stage breast cancer or patients with one to three positive axillary lymph nodes (LN) where the assay stratified patients into low- and high-

genomic risks groups to determine the choice of therapy. Another assay is Prosigna which was originally designed for simple identification of molecular subtypes of breast cancer that based on specific biological characteristics such as their hormone and HER2 signalling pathway, nuclear proliferation score and markers of basic phenotype. Subsequently it evolved to evaluate the relapse risk or early breast cancer and was approved by the US FDA in 2013. Generally, Prosigna added additional prognostic information to clinical variables in hormone receptor -positive (HR+) early-stage node-positive (N+) and N-. EndoPredict is a 12-gene risk score which was developed and validated for classification of HR+, N- early breast cancer into low-risk or high-risk for distant recurrence.

POLICY QUESTIONS

3.1 Is molecular profiling assay as part of early breast cancer management, beneficial to predict the recurrence risk of early breast cancer?

3.2 Should the molecular profiling assay be part of early breast cancer management in Ministry of Health (MOH)?

OBJECTIVES

4.1 To assess the relative effectiveness and safety of different types of molecular profiling and subsequent management in breast cancer.
(As a result of this, decision to give or not to give chemotherapy will determine patient outcomes such as mortality, and quality of life [QoL]).

4.2 To assess the economic implication, social, ethical, and organisational aspects related to molecular profiling of early breast cancer.

The following **research questions** will be addressed:

4.1.1 What is the accuracy/performance of different types of molecular profiling assay in predicting recurrence risk?

4.1.2 Is molecular profiling assay cost-effective?

4.1.3 Which is the best molecular profiling assays in terms of accuracy and cost-effective?

4.1.4 What is the social, ethical, and organisational implication/impact related to molecular profiling?

4.1.5 Which population can benefit from the molecular profiling assays?

METHODS

Literature search was developed by the main author and *Information Specialist* who searched for published articles pertaining to molecular profiling assays in breast cancer. The following electronic databases were searched through the Ovid interface: Ovid MEDLINE® and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions® 1946 to June 2022, EBM Reviews - Health Technology Assessment (4th Quarter 2016), EBM Reviews - Cochrane Database of Systematic Review (2005 to January 2022), EBM Reviews - Cochrane Central Register of Controlled Trials (June 2022), and EBM Reviews - NHS Economic Evaluation Database (4th Quarter 2016). Parallel searches were run in PubMed, US FDA and INAHTA database. Search was limited to articles in English and in human.

RESULTS

PART A: SYSTEMATIC REVIEW

A total of **297** records were identified through the Ovid interface and PubMed while **2** were identified from other sources (references of retrieved articles). Following the removal of **138** duplicates and irrelevant titles, **161** titles were found to be potentially relevant and abstracts were screened using the inclusion and exclusion criteria. Of these, **50** relevant abstracts were retrieved in full text. After reading, appraising and applying the inclusion and exclusion criteria to the **50** full text articles, **16** full text articles were included. The 16 studies consisted of **three** systematic reviews (SRs), **seven** retrospective cohorts, and **six** economic evaluation studies (consisted of one SR, one budget impact analysis and four cost-effectiveness studies). Three prospective cohort studies on Oncotype DX test and MammaPrint (TAILORx, RxPONDER and MINDACT) were already discussed in the included SRs and will not individually reported in this assessment.

EFFECTIVENESS

Individual Findings

Oncotype DX – Recurrence Score (RS)

The Oncotype DX test showed an excellent prognostic ability in patients with HR+/HER2- N0 breast cancer. One of the included studies showed a statistically significant difference in the six-years distant recurrence free survival (DRFS) rate of 94.4%, 96.9% and 85.1% between low-, intermediate- and high-genomic risk groups respectively ($p < 0.001$). The five-year overall survival (OS) difference between the groups to the Recurrence Score (RS) classification was

99% for low- and intermediate-risk and 92%-94% for high-risk population. RS was correlated with the effect of chemotherapy in the three risk groups in terms of 10-years DRFS which was significantly increased in the high-risk group receiving chemotherapy compared to the low-RS group.

Within HR+/HER2- N1 breast cancer subgroup, the six-years DRFS with Oncotype DX-Recurrence score were 92.3%, 85.2%, and 71.3% in low-, intermediate and high-genomic risk groups respectively. The interaction between RS and clinical benefit of chemotherapy in the lymph node positive subgroup was significant for the first five-years after treatment.

The included studies also reported that the Oncotype DX test led to changes in treatment recommendations. The percentage of changes recommendations in most of the included studies ranges between 21% to 74% in either escalation or de-escalation of chemotherapy. Specifically, the de-escalation of chemotherapy to no chemotherapy ranged between 6.1% to 74%.

MammaPrint

When compared with clinical parameters-only assessment, MammaPrint reclassified the risk category of patients with good clinical prognostic factors to either low-risk or high-risk patients. In addition, MammaPrint also significantly predict the chemotherapy outcome and prognostic value in both LN- and LN+ tumours.

Addition of MammaPrint assay test result to the clinical-pathological assessment, led to changes in treatment recommendations. The overall changes were between 18% to 40%, with decision from chemotherapy to no chemotherapy ranges between 2% and 32%.

EndoPredict – EP score and EPclin

In LN+ breast cancer of pre-menopausal women, the EndoPredict test reported a distant metastasis free survival (DMFS) at 10-years at 93% in low-risk group compared to 67% in high-risk group ($p<0.0001$).

Prosigna/PAM50 – Risk of Recurrence (ROR)

The included studies reported that the Prosigna discriminated between low-risk and high-risk patients very well.

Correlation and Concordance between Assays

There was no prospective head to head trial comparing each of the assays retrieved. Only a few studies looked at the correlation and concordance between the available assays. These studies were included in this HTA report. Overall, each molecular profiling assay had either very week correlation or no correlation among each other.

Oncotype DX versus MammaPrint

Oncotype DX was initially utilised among stage I node negative but later included node positive patients in the RxPONDER study. Both assays were associated with a significant decrease rate of chemotherapy administration with profiling versus without molecular profiling test (24.5% versus 37.2%; $p < 0.001$).

Oncotype DX versus EndoPredict

There was positive Pearson correlation between EndoPredict and Oncotype DX, $r = 0.65$ with 76% concordance between risk categories. However, this study had only a small sample size, $n = 34$ hence the results have to be interpreted with caution.

Oncotype DX versus Prosigna

Based on Spearman correlation coefficient, Oncotype DX and Prosigna had very weak positive correlation ($r_s = 0.08$). Both assays also showed weak correlation when applied to post-menopausal women; $r_s = 0.276$, $p = 0.013$.

MammaPrint versus EndoPredict

Although MammaPrint to EPclin showed significant association in the overall population (included all risk cases), both assays failed to show a significant association amongst the high-risk subgroup ($p = 0.294$, $\kappa = 0.15$, 95% CI -0.089 – 0.39).

Molecular Profiling Assays versus Clinical-Pathological Model

This study found that patients with larger tumour size (>20mm), Allred PR expression of 0-4 and higher-grade tumours (grade III) had higher likelihood ratio (LR) of high-genomic risk; odds ratio 3.84, 95% CI 1.84 – 6.98 ($p < 0.001$), odds ratio 3.46; 95% CI 1.76 – 6.82 ($p < 0.001$) and odds ratio7.24; 95% CI 3.82- 13.70 ($p < 0.001$), respectively. This confirms the ineligibility of grade 3 tumours to be tested with genomic assays.

SAFETY

No safety issue related to molecular profiling assays in breast cancer was retrieved.

ORGANISATIONAL

There were two guidelines related to the use of molecular profiling in management of early-stage breast cancer retrieved. The most recent guideline was published in 2022 by Ontario Health (Cancer Care Ontario) Health Program in Evidence-Based Care (PEBC) in Canada and another guideline was published in 2018 by the National Institute for Health and Care Excellence (NICE) in the United Kingdom. The Ontario guideline was intended for clinician and policymakers who are involved in the diagnosis and treatment of breast cancer. As for NICE guideline, molecular profiling is used to guide adjuvant chemotherapy decision in early breast cancer. Generally, both guidelines recommended the used of molecular profiling as an option to guide systemic therapy or chemotherapy decision in patients with ER+ HER2-ve early-stage breast cancer.

SOCIAL

Generally, not many patients are aware about molecular profiling assays and their utility in breast cancer management. However, after being introduced and having a personal experience with the assays, most of the patients expressed higher confidence with the final treatment recommendations.

ECONOMIC EVALUATIONS

One SR on economic evaluations of Oncotype DX reported that Oncotype DX had an ICER of ≤$100,000 per QALY. The SR also evaluated the probability of industrial funded studies which might influence the outcome of the economic evaluations. Fortunately, in both industrial funded or non-funded studies, the Oncotype DX test was associated with cost-saving, with favourable ICERs of US$900 versus US$3,100 per QALY. In another SR, if patient's outcome is being considered, any use of molecular profiling assays was cost effective in 90% of the economic evaluation studies, regardless of the type of assays used. On the other hand, when comparing N- and N+ breast cancer, the estimated QALYs gained was larger in N- (on average 0.24 versus 0.07 QALYs) than N+ patients. In Germany, the Oncotype DX was cost saving with no negative impact on mortality when compared with EndoPredict and MammaPrint; as the average saving per patient was 2,500€ and 1,936€ when compared to EndoPredict and MammaPrint respectively. Meanwhile, the Canadian public healthcare system view that, the

addition of molecular profiling assays into clinicopathological predictors to guide chemotherapy decision was cost-effective. In the UK study, Prosigna was deemed the preferred assay for further research. However, in the sensitivity analysis, Oncotype DX was the favoured assay on the basis of its expected cost-effectiveness followed by Prosigna. In Spain, Oncotype DX and MammaPrint played a significant role in treatment management of patients with early-stage breast cancer and both assays were cost-saving and highly cost-effective at national health care system and societal perspective; 13,920€ (95% CI 11,697€ - 12,218€) and 32,793€ (95% CI 28,432€ - 37,827€), respectively. In Turkey, the Oncotype DX was found to be cost-effective at national health care perspective with improvement in QoL and may be introduced for routine clinical practice among early breast cancer patients. The ICERs was estimated to be $7,207.9 per QALY gained and 5,720.6 per LY gained for Oncotype DX versus current clinical practice in Turkey.

PART B: ECONOMIC EVALUATION

Objectives

The general objective of this economic evaluation was to assess the cost benefit of using new molecular profiling assays in guiding decision making on chemotherapy treatment for early HR-positive HER2-negative breast cancer patients.

The specific objectives were to estimate the savings associated with the usage of new molecular profiling assays compared to conventional clinical risk prognostic tools in decision making on chemotherapy for HR-positive HER2-negative node negative (N0) as well as node positive (N1-3) in early breast cancer patients; and to estimate the budget implicated for the population that would benefit from the cost savings.

Methods

A decision tree model was developed with Microsoft 365 Excel Workbook® to estimate the costs and benefit of using molecular profiling assays for chemotherapy guidance in early HR-positive HER2-negative breast cancer compared with using conventional non-genetic risk prognostic tools (St Gallens classification, PREDICT online, Adjuvant! Online) alone. The perspective taken was from the Ministry of Health perspective.

The population included in the simulation cohort were the HR- positive, HER2- negative early breast cancer with either LN- negative (No node involvement) or LN-positive (one to three node involvement) who have undergone surgery.

Based on the systematic review and meta-analysis conducted in this HTA report earlier, molecular profiling assays (regardless the type of assays) was cost effective in 90% of economic evaluation studies, with estimated QALYs gained larger in the node-negative group. Regardless of lymph node status, Oncotype DX and MammaPrint was able to predict the potential benefit to be seen with omission or administration of chemotherapy. For the purpose of this cost benefit analysis, the Oncotype DX and MammaPrint tests were simulated in the model as the locally available interventional gene expression profile assays, and the comparator was the conventional non-genetic risk prognostic tools.

The short-term outcome was measured as cost benefit from chemotherapy averted.

Model Structure

The model structure was constructed following a literature review, and consultation with an expert committee which consisted of multidisciplinary experts namely clinical oncologists, breast and endocrine surgeons, pathologists, radiologists, health economists, public health physicians and pharmacists. This economic evaluation was designed from the Ministry of Health (MOH) perspective.

Model Estimation

The epidemiological and disease-related data were obtained from local sources of data whenever available, or literature review when local data was not available. The proportion of patients in each risk level is taken from literature review, while the cost of treatment was from local institution data. The hypothetical cohort was derived from mixed local registry data and literature review.

Results and Conclusion

From the decision analytic modelling that has been conducted, for a hypothetical cohort of 3,500 patients simulated, usage of Oncotype DX was cost saving in the intermediate risk of recurrence group, in both lymph node positive and lymph node negative patients. In LN-negative cohort, there is an estimated cost savings of MYR 10,703,458.56 for those with intermediate risk of recurrence, and in the LN-positive cohort, there was an estimated cost

savings of MYR 4,447,623.36 in those profiled as having intermediate risk of recurrence. However, incremental cost was valued at MYR 17,341,739.76 in the LN-negative cohort and MYR 7,540,934.88 in the LN-positive cohort. An overall incremental cost of MYR 24,882,674.64 was estimated if a blanket testing of all eligible patient population was performed.

For the cohort of 3,500 patients simulated, usage of MammaPrint gave an overall incremental cost of MYR 67,395,212.24 in LN-negative patients and MYR 28,869,914.40 in LN-positive patients. This resulted in an overall incremental cost of MYR 96,265,126.64 if all eligible 3,500 were tested with MammaPrint regardless of LN status and risk stratification.

In conclusion, both Oncotype DX and MammaPrint incurred incremental cost if they are utilized to test the whole eligible patient population. However, cost savings of approximately MYR 15,151,081.92 can be achieved with the usage of Oncotype DX in both intermediate risk of recurrence LN-negative group and LN-positive group of 880 patients averting chemotherapy. Therefore, maximal cost savings and potential benefits in averting chemotherapy and chemotherapy complications may be achieved if targeted testing was performed using Oncotype DX in the intermediate risk of recurrence group. The budget implications to procure Oncotype DX assays for 1,574 patients would be MYR 23,610,000.00.

CONCLUSION

Molecular profiling assays are significantly effective in prognosticating between low-risk and high-risk of recurrence among patients with HR+/HER2-ve early-stage breast cancer. However, further assessment is required in terms of predicting of chemotherapy benefit, Oncotype DX and MammaPrint are able to predict the chemotherapy benefit regardless of lymph-nodes status. Individual prospective assays are available but there are not head to head prospective study to compare between the assays. Retrospective study looking at the association and correlations between the assays are limited in number and has small sample size (<100). Each assay had poor to weak association with each other and should not be used interchangeably. Overall, LN- and low-risk early breast cancer patients might benefit more from molecular profiling assays. Economically wise, the molecular profiling assays were cost-effective compared to conventional method and Oncotype DX was the most commonly used.

In economic evaluation, both Oncotype DX and MammaPrint incurred incremental cost if utilized for testing the whole eligible population. However, cost savings of approximately MYR 15,151,081.92 can be seen with usage of Oncotype DX in both intermediate risk of recurrence LN-negative and LN-positive breast cancer patients with 880 patients who averted chemotherapy. Therefore, maximal cost savings and potential benefits in averted chemotherapy with its complications may be achieved if targeted testing was performed using Oncotype DX in the intermediate risk of recurrence group. The budget implications to procure Oncotype DX assays for 1,574 patients would be MYR 23,610,000.00.

The sensitivity analysis showed that overall cost savings can be achieved if the price of Oncotype DX is reduced to 50% of the quoted price, giving a total accrued cost savings of MYR 1,367,325.36. If price negotiation can be done, a minimum reduction of 50% of the Oncotype DX price may potentially offer eligible population greater access to Oncotype DX assay regardless of LN status or risk. The budget required for procurement of Oncotype DX assay for 3,500 patients with reduction to 50% of the quoted price is MYR 26,250,000.00

POLICY RECOMMENDATION

Molecular profiling assays has a role in discriminating recurrence risk in HR+/HER2- early-stage breast cancer patients. Oncotype DX may be recommended in management of HR+/HER2- early breast cancer with the maximal potential benefit in the intermediate risk of recurrence group with purchasing price negotiation.

TABLE OF CONTENTS

ABBREVIATION

AoL	Adjuvant! Online
BCSS	Breast cancer specific survival
CBO guideline	Dutch Institute for Healthcare Improvement guidelines 2004
DFS	Disease free survival
DRFS	Distant recurrence free survival
DMFS	Distant metastases free survival
ER+/ER-	Oestrogen Receptor positive / Oestrogen Receptor negative
EVPI	Expected value of perfect information
EVSI	Expected value of sample information
EVPPI	Expected value of perfect parameter information
FISH	Fluorescence In Situ Hybridization
HTA	Health Technology Assessment
HR	Hazard Ratio
HR+ / HR-	Hormone Receptor positive / Hormone Receptor negative
ICER	Incremental cost-effectiveness ratio
IHC	Immunohistochemistry
LN- / LN+	Lymph node negative / Lymph node positive
LR+ / LR-	Positive likelihood ratio / Negative likelihood ratio
LYs	Life Years
MaHTAS	Malaysian Health Technology Assessment Section
MINDACT Trial	Microarray in Node-Negative and 1 to 3 Positive Lymph Node Disease May Avoid Chemotherapy Trial
MNCR	Malaysia National Cancer Registry
MOH	Ministry of Health
N+ / N-	Node positive / Node negative
NCCN	National Comprehensive Cancer Network
NICE	UK National Institute for Health and Care Excellence's
NPI	Nottingham Prognostic Index
OS	Overall Survival
PR+ / PR-	Progesterone Receptor positive / Progesterone Receptor negative
QALYs	Quality adjusted life years
QoL	Quality of life
RCT	Randomised controlled trial
ROR	Risk of Recurrence
RS	Recurrence Score
TAILORx	Trial Assigning Individualised Options for Treatment
US FDA	United States Food and Drug Administration
WHO	World Health Organization
WSG PlanB Trial	West German Study Group PlanB Trial

1.0 BACKGROUND

The most recent Malaysian National Cancer Registry (MNCR) 2012-2016 showed an increasing trend of breast cancer cases from 18,206 (MNCR 2007 – 2011) report to 21,634 (current MNCR 2012 – 2016).[1,2] According to Globocan 2020, 17.3% (8,418) of new breast cancer cases was reported in Malaysia in 2020.[3] Approximately 48% of breast cancer cases in Malaysia were diagnosed late with age standardised incidence rate (ASR) of 34.1 per 100,000 populations.[4] Thus, as an incentive to encourage women to undergo screening, the government provides subsidised mammograms through the Ministry of Women and Family Development (LPPKN) and state government programmes. Unfortunately, the level of breast cancer screening utilisation in Malaysia is low probably influenced by educational level, socioeconomic status, cultural perception and beliefs of women and community.[1]

There are several risk factors of breast cancer that can be divided into non-modifiable and modifiable. The one that non-modifiable and currently play an important role in treatment choice is genetic factor or genetic mutation.[5] According to the Clinical Practice Guideline (CPG) Management of Breast Cancer (3rd Edition), there are advancements in screening method, early prognosis even in treatment modalities over the years. This included molecular subtyping that is becoming popular and considered as an important factor in determining treatment response. Advances in molecular biology and pharmacology aids in better understanding of breast cancer, enabling the design of effective therapy to target the cancer.[6]

In general, molecular profiling is a scientific approach that compare different types of tissues at a molecular level (DNA, mRNA or protein) on a global scale.[7] The molecular profiling test is a genomic technology use in predicting individual patient's prognosis by interpreting the expression pattern of a panel of specific tumour-related genes.[8] The genomic test looks at all the genes and examine how the genes interact and affect health.[9] The transcription of specific set of genes is used as a surrogate marker for metastatic potential. The pattern of gene expression and the specific gene expression threshold levels can identify the tumours with more aggressive biology, thereby quantifying the risk of recurrence more accurately for either less or more aggressive treatment.[8] As for genetic testing, it is designed to detect a single gene mutation associated with specific cancer such as BRCA1 and BRCA2 mutations that associated with breast and ovarian cancer.[9]

Three subtypes of breast tumours with different biologic behaviours were discovered using the traditional ImmunoHistoChemistry (IHC) techniques: hormone-receptor-positive, triple negative, and Human Epidermal Receptor (HER) 2/neu-positive breast cancers. All of these subtypes have distinct natural histories, which require different management approach. On the other hand, the availability of expression profiling and hierarchical clustering enabled to identify the additional subtypes. Breast cancer comprises of at least 7 different biologic subtypes. They include luminal A, luminal B, luminal C, HER2-enriched, basal-like, claudin-low, and normal breast-like.[8] As an example, patients who are identified with early-oestrogen receptor-positive (ER+) lymph node negative (LN-) breast cancer are likely to have higher risk of recurrence. Meanwhile, patients who are identified as low risk may be avoiding possible unnecessary treatment as well as the short or long-term side effects that associated with chemotherapy.[5]

Thus, the molecular profiling tests aim to improve the use of chemotherapy in breast cancer by improving the categorisation of patients in accordance with risk and the identification of those patients who will gain most benefit from chemotherapy.[10] There are several commercially available molecular profiling tests including Oncotype DX, Prosigna (Predictor Analysis of Microarray 50 [PAM 50]), EndoPredict and MammaPrint. The tests are typically performed after surgery once hormone and lymph node status are known including other information such as tumour size and tumour grade.[11]

Reasons for request

More demands are coming from patients and clinicians to use gene assays profiling as part of the management of early breast cancer. However, in-depth knowledge of the different assays, their usefulness, cost-effectiveness is not readily available for a sound decision making process by clinicians for the individual patient(s).

2.0 TECHNICAL FEATURES

The Oncotype DX, MammaPrint, Prosigna and EndoPredict test are four molecular profiling assays which already approved by the Food and Drug Administration (FDA) agency and European Medicines Agency (EMA) for early-stage breast cancer to predict recurrence risk and guide adjuvant chemotherapy decisions.

2.1 Four Common Types of Molecular Profiling Assays

2.1.1 Oncotype DX

Oncotype DX is a 21-gene expression assay which was initially developed for women with lower grade, small tumours (<5cm), N-, ER+ breast cancer. It was developed and validated from combined cohort of breast cancer patients. During the development process the researchers found that the Recurrence Score (RS) consistently and independently predicted recurrence free survival of the patients and could be used as continuous function to predict outcome in patients treated with hormone therapy. The patients are categorised into three risk stratifications based on the RS score; low-risk score (score <18), intermediate-risk score (score 18-30) and high-risk score (score >30). However, there is another threshold from TAILORx trial which is; low-risk score (score <11), intermediate-risk score (score 11-25) and high-risk score (score >25).[11,12, 13]

2.1.2 MammaPrint

MammaPrint is a 70-gene assay which was previously generated for patients with LN-early stage breast cancer where the assay stratified patients into low- and high-genomic risks groups to determine a choice of therapy. Later, patient's subgroup was expanded among one to three positive axillary lymph nodes. Patients are classified by calculating the correlation coefficient between a patients' 70-gene expression levels and the average good-prognosis expression profile. If the correlation coefficients exceed 0.4, the patients are classified as having a good prognosis; if not, the patients are classified as having poor prognosis.[11, 12, 13]

2.1.3 Prosigna

Prediction Analysis of Microarray-50 (PAM50, PAM50-ROR Score or Prosigna) which was included 'intrinsic breast cancer subtypes' consisted of luminal A, luminal B, HER2-enriched and basal-like subtypes among node-negative and node-positive patients. Originally it was designed for simple identification of molecular subtypes of breast cancer that based on specific biological characteristics such as their hormone and HER2 signalling pathway, nuclear proliferation scores and markers of basic phenotype. Later, Prosigna was developed to evaluate the relapse risk. The risk of recurrence (ROR) is classified into four types based on different factors. The ROR types and the recurrence risk score are; ROR combined with subtypes (ROS-S; low-risk <24, intermediate risk 24-53), high-risk >53), ROR combined with subtypes and proliferation (ROR-P; low-risk <12, intermediate-risk 12-53; high risk >53), ROR

combined with subtypes and tumour size (ROR-T; low-risk <29, intermediate-risk 29-65, high-risk >65) and ROR combined with subtypes, proliferation, tumour size (ROR-PT; low-risk <18, intermediate-risk 18-65, high-risk >65). Generally, Prosigna added additional prognostic information to clinical variables in ER/PR+ early-stage node-positive and node-negative.[11,12,13]

2.1.4 EndoPredict

EndoPredict is a 12-gene risk score which was developed and validated for classification of breast tumour into low-risk and high-risk of distant recurrence in ER/PR+, N- early breast cancer. The EndoPredict risk score was EP score range from 0 to 15 dividing the risk into two groups including low-risk (<5) and high-risk (EP >5). Another EndoPredict score was EPclin score which is the combination of EP score and two clinical factors (nodal status and tumour size) and the predictive power exceeds EP score alone.[11,12, 13] Table 1 summarised the information regarding the four assays.

Table 1: Molecular profiling test used for chemotherapy decision-making in ER-positive, ERBB2 (HER2)-negative breast cancer

Information	MammaPrint	Oncotype DX	Prosigna	EndoPredict
				
Number of genes	70	21	50	11
Method	DNA microarray	RT-PCR	Nanostring	RT-PCR
Tissue sample type	Frozen/FFPE	FFPE	FFPE	FFPE
Test results	High or low risk +subtype	High, Intermediate or low risk	High, intermediate or low risk +subtype	High or low risk
Clinical Indication (according to EGTM)	Predicting prognosis and guiding decision-making regarding chemotherapy for women with ER+/HER2- EBC, LN- or LN+ (1-3)	Predicting prognosis and guiding decision-making regarding chemotherapy for women with ER+/HER2- EBC, LN- or LN+ (1-3)	Predicting prognosis and guiding decision-making regarding chemotherapy for women with ER+/HER2-ve EBC, LN- or LN+ (1-3)	Predicting prognosis and guiding decision-making regarding chemotherapy for women with ER+/HER2- EBC, LN- or LN+ (1-3)

Information	MammaPrint	Oncotype DX	Prosigna	EndoPredict
				
Prospective validation trial(s)	MINDACT (positive)	TAILORx (positive) and RxPONDER (ongoing)	OPTIMA (ongoing)	None
Regulatory approval	EMA, FDA	EMA, FDA	EMA, FDA	EMA, FDA
Original validation set	Developed in young patients (aged <55 years) who had not received therapy after surgery	Developed in patients who had received tamoxifen only in the NSABP B-20 and B-14 trials	Postmenopausal patients in the training and development sets received heterogeneous treatment	Developed in postmenopausal patients who had received endocrine therapy only in the ABCSG-6 and -8 trials

*Table adapted from WHO BlueBooks[14]
FFPE: Formalin-fixed, paraffin-embedded; EGTM: European Group on Tumour Markers
Assay's Images from: https://www.breastcancer-news.com, https://www.sciencewerke.com/all_products/myriad-endopredict/, https://www.medgadget.com/2008/12/mammaprint_identifies_low_risk_her2_patients.html, https://www.businesswire.com/news/home/20140805006562/en/NanoString-Technologies-Receives-Market-Approval-From-the-Australian-Therapeutic-Goods-Administration-for-Its-Prosigna-Breast-Cancer-Prognostic-Gene-Signature-Assay

2.2 Criteria of breast cancer patients eligible for Molecular Profiling assays test

According to the expert committees, ER+, HER2- luminal tumours is the most common breast cancer subtype (around 50-70%) of all breast cancers. After initial surgery, patients with low clinico-pathological risk; tumour size of less or equal to 5 cm (pT1-2); node negative or lymph node 1-3 positive (pN1) Grade 1-2, with low nuclear proliferation factor (Ki67) and expressing ER positive and HER2- disease usually do not require chemotherapy. However, a small subgroup of patients may harbour gene expression that either categorise them into having high-risk tumours requiring chemotherapy or low risk tumours which can avoid chemotherapy. Listed here are criteria of breast cancer patients who are eligible and beneficial to undergo molecular profiling test. The patients should have:[11, 15]

i. Early-stage invasive breast cancer (Stage I to II breast cancers that are surgically operable

- ER+/HER2- N0 early-stage breast cancer that is under consideration for adjuvant chemotherapy
- ER+/HER2- N+ early-stage breast cancer that is under consideration for adjuvant chemotherapy

ii. Already or willing to do tumour removal surgery (because the test requires tumour sampling)

iii. Not having chemotherapy yet

iv. Do not have evidence of locally recurrent or distant metastatic disease with pT1-T3 or pN0-pN1a based on surgical pathologic staging

To better stratify the risk by providing a more accurate indicators of recurrence risk and guide treatment decisions (which patients may derive benefit or which patients can avoid chemotherapy) researchers had focused on the biological tumour characteristics using molecular profiling assays. At present, molecular profiling assay is not indicated for these patient subgroups with at least one of these characteristics:

i. HER2 positive breast cancer

ii. Triple negative breast cancer

iii. Tumour Grade 3

iv. High Ki67 > 20%

v. Tumour more than 5 cm

vi. Four or more lymph node postitive

vii. Hormone receptor positive HER2 negative early breast cancer with very low clinico-pathological risk tumours less or equal to 1 cm, node negative (pT1a–b, pN0), Grade 1 and high ER positive HER2- disease.

2.3 Prognosis and predictive of molecular profiling assays

Main purpose of most of the molecular profiling assays is to determine whether a tumour has a high or low risk for recurrence. The assays evaluate the intrinsic molecular characteristics of a tumour to prognosticate behaviour, some of the assays able to predict a treatment benefit. The genes used to ascertain the predicted risk are differing among assays. Each individual assays uses different scoring system and the results may not be directly comparable, although the results of the risk category between assays are similar. No matter what, the risk scores of any molecular profiling assays should be interpreted with caution and the decisions should be made after considering other clinical, pathological or patient-related factors.[15]

3.0 POLICY QUESTIONS

3.1 Is molecular profiling assay as part of early breast cancer management, beneficial to predict the recurrence risk of early breast cancer?

3.2 Should the molecular profiling assay be part of early breast cancer management in Ministry of Health (MOH)?

4.0 OBJECTIVES

4.1 To assess the relative effectiveness and safety of different types of molecular profiling and subsequent management in breast cancer.
(As a result of this, decision to give or not to give chemotherapy will determine patient outcomes such as mortality, and quality of life [QoL]).

4.2 To assess the economic implication, social, ethical, and organisational aspects related to molecular profiling of early breast cancer.

The following **research questions** will be addressed:

4.1.1 What is the accuracy/ performance of different types of molecular profiling assay in predicting recurrence risk?

4.1.2 Is molecular profiling cost-effective?

4.1.3 Which is the best molecular profiling assays in terms of accuracy and cost-effective?

4.1.4 What is the social, ethical, and organisational implication/ impact related to molecular profiling?

4.1.5 Which population can benefit from the molecular profiling assays?

5.0 PART A: SYSTEMATIC REVIEW OF LITERATURE

5.1 METHODS

5.1.1 Literature Search strategy

Literature search was developed by the main author and an *Information Specialist* who searched for published articles pertaining to molecular profiling assays in breast cancer. The following electronic databases were searched through the Ovid interface: Ovid MEDLINE® and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions® 1946 to

June 2022, EBM Reviews - Health Technology Assessment (4th Quarter 2016), EBM Reviews - Cochrane Database of Systematic Review (2005 to January 2022), EBM Reviews - Cochrane Central Register of Controlled Trials (June 2022), and EBM Reviews - NHS Economic Evaluation Database (1st Quarter 2016). Parallel searches were run in PubMed, US FDA and INAHTA database. There was no limitation in language, however, in the end only articles in English were included. Year of publication was limited from year 2000 to 2022 and only human study were included. Detailed search strategy is as in **Appendix 3**. The last search was performed on 30th June 2022. Additional articles were identified from reviewing the references of retrieved articles.

5.1.2 Study selection

Two dedicated reviewers independently screened the titles and abstracts against the inclusion and exclusion criteria as shown below and evaluated the selected full-text articles for final article selection. Disagreement was resolved by discussion.

Inclusion Criteria

a.	**Population**	Early-stage breast cancer lymph node status *(LN-positive [LN+, n0, n1], LN-negative [LN-])*, and receptor status *(ER-positive [ER+], HER2-negative [HER2-])* and pre- and post-menopausal women
b.	**Intervention**	Molecular profiling / gene expression profiling (GEP) / tumour profiling test (Oncotype DX, MammaPrint, EndoPredict, Prosigna and immunochemistry 4 (IHC4))
c.	**Comparator**	i. Comparing among molecular profiling assays ii. None/Usual care
d.	**Outcomes**	i. Effectiveness: Prognostic performance (Recurrence Score [RS], Risk of Recurrence [ROS] score), prediction of systemic treatment benefit, breast cancer-related mortality, quality of life (QoL) ii. Safety: adverse events, complications iii. Economic implications: cost-effectiveness, cost-utility, cost-benefit analysis iv. Potential psychological and behavioural harms and benefits of test results v. Training requirements or learning curve

e.	**Study design**	HTA reports, systematic review with/out meta-analysis, randomised controlled trial (RCT), cohort, diagnostic, cross-sectional, case-control, economic evaluation studies
f.	Full text articles published in English	

Exclusion Criteria:

a.	**Study design**	Animal study, laboratory study, case report, case series, narrative review
b.	Non-English full text articles	

5.1.3 Critical appraisal of literature/ assessment of risk of bias

The risk of bias or quality assessment (methodology quality) of all retrieved literatures was assessed depending on the type of the study design; checklist of National Collaborating Centre for Methods and Tools (ROBIS)[16] for Systematic Review and Meta-analysis, Cochrane Risk of Bias Tool (RoB 2) for Randomised Controlled Trials[17], and Critical Appraisal Skill Programme (CASP)[18] for Observational and Economic Studies. All full text articles were graded based on guidelines from the *U.S. / Canadian Preventive Services Task Force* (**Appendix 1**).[19]

5.1.4 Analysis and synthesis of evidence

Data extraction strategy

Data were extracted from included studies by a reviewer using a pre-designed data extraction form (*Evidence Table* as shown in **Appendix 4**) and checked by another reviewer. Disagreements were resolved by discussion and the extracted data was also presented and discussed with the *Expert Committee*. The data extracted was as follows:

i. Details of methods and study population characteristics
ii. Detail of intervention and comparators
iii. Details of individual outcomes specified

Methods of data synthesis

Data on the effectiveness, and cost-effectiveness associated with molecular profiling assays were presented in tabulated format with narrative summaries. No meta-analysis was conducted for this review due to high heterogeneity especially in the characteristics of breast cancer populations, and the difference between the assays itself.

5.2 RESULTS

5.2.1 Selection of Included articles

An overview of the systematic search and selection of the studies are illustrated in **Figure 2.** A total of **297** records were identified through the Ovid interface and PubMed while **2** titles were identified from other sources (references of retrieved articles). Following the removal of **138** duplicates and irrelevant titles, **161** titles were found to be potentially relevant and abstracts were screened using the inclusion and exclusion criteria. Of these, **50** relevant abstracts were retrieved in full text. After reading, appraising and applying the inclusion and exclusion criteria to the **50** full text articles, **16** full text articles were included. **Thirty-four** articles were excluded as those primary studies were already included in the systematic reviews and HTA (n = 13), irrelevant objective and scope of study (n = 11), other types of molecular profiling test (n = 2), small sample size (n = 1) and narrative reviews (n = 6). The excluded articles were listed as in **Appendix 5**.

The 16 full text articles which were finally selected in this review comprised of **three** systematic reviews[20-22], **seven** cohort studies[23-29], and **six** economic evaluation studies[30-35] (consisted of one SR, one budget impact analysis and four cost-effectiveness studies). Three prospective cohort studies on Oncotype DX test and MammaPrint (TAILORx, RxPONDER and MINDACT) were already discussed in the included SRs and will not individually reported in this assessment.

All studies included were published in English language between 2013 and 2021 and were conducted in the United States, United Kingdom, Canada, Japan, Turkey, German, Spain and China.

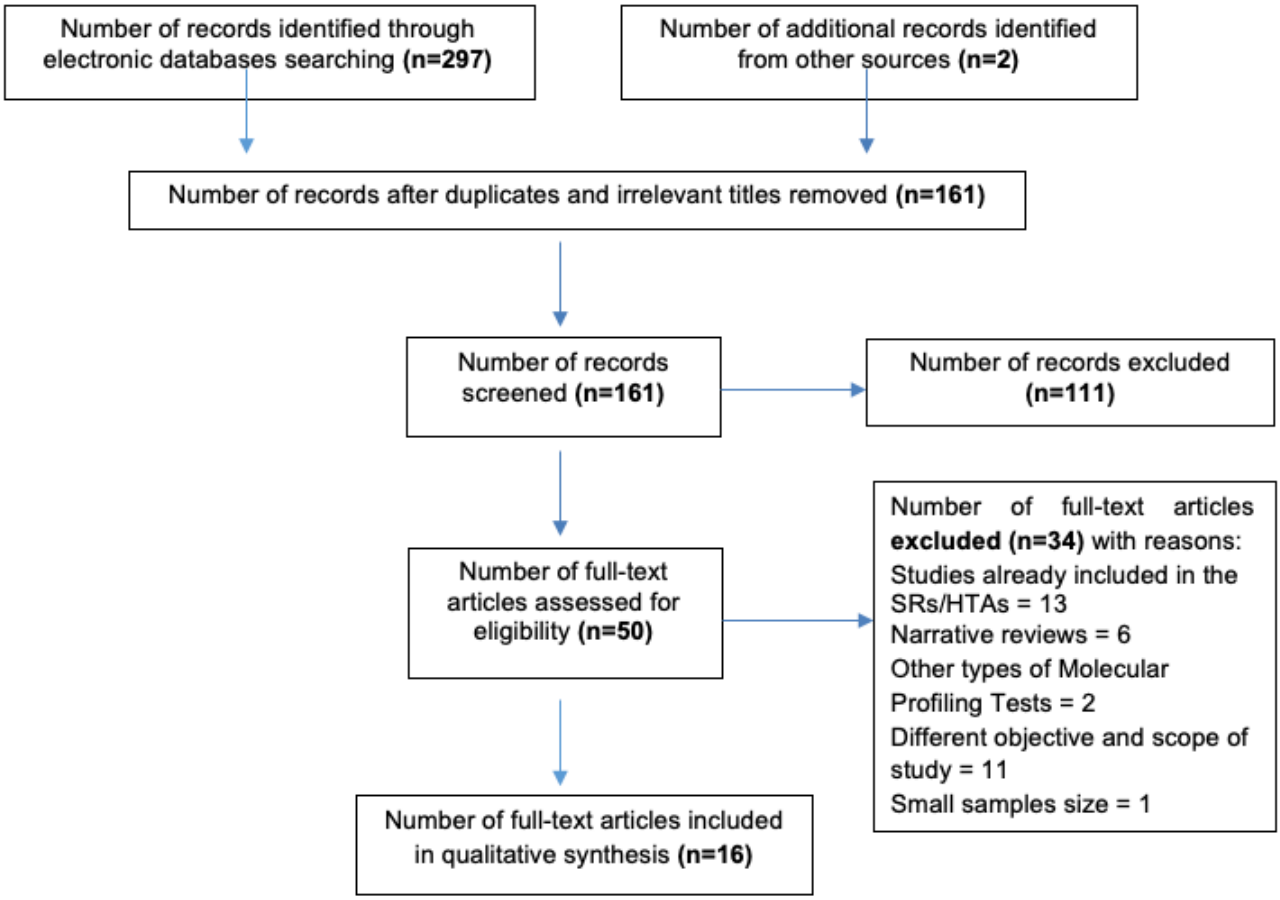


Figure 2: Flow chart of retrieval of articles used in the results

5.2.2 Quality assessment / risk of bias

Risk of bias was assessed using Risk of Bias in Systematic Reviews (ROBIS) for systematic review, and Critical Appraisal Skill Programme (CASP) checklist for observational study. These assessments involved answering a pre-specified question of those criteria assessed and assigning a judgement relating to the risk of bias.

Risk of bias assessment for included systematic review

Three studies were included in this assessment. Two SRs[20, 22] were judge to have an overall low-risk of bias meanwhile one SR by Blok EJ. et. al.[21] was high-risk of bias (**Figure 3.1**). The high-risk bias was given to the Blok EJ. et. al. because the authors did not performed risk of bias assessment on the included studies.

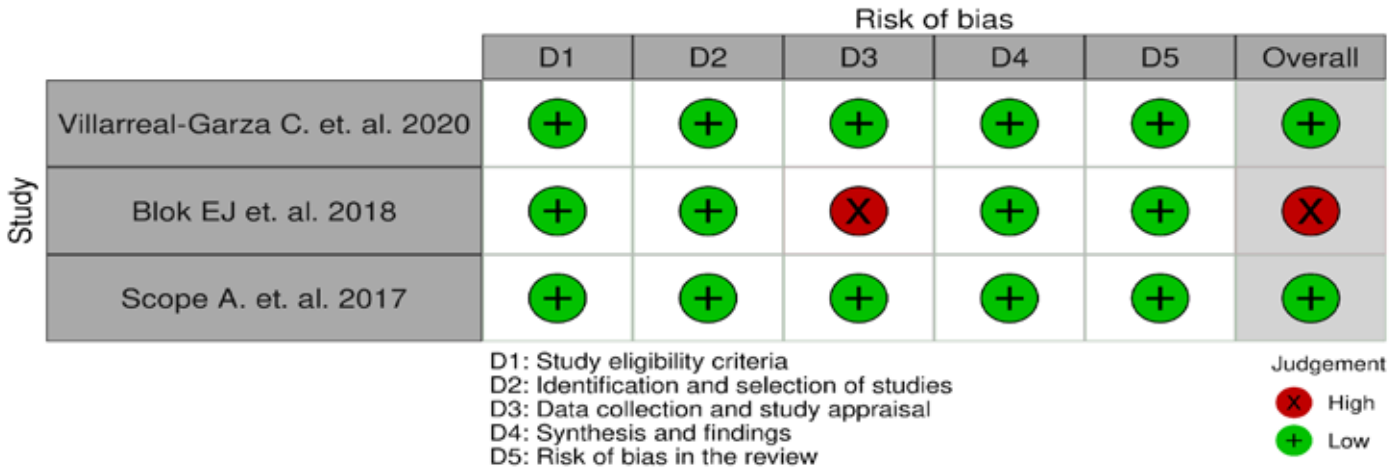


Figure 3.1: Summary of risk of bias assessment for systematic review using ROBIS

Risk of bias assessment for included cohort studies

Seven cohort studies were included in this risk of bias assessment (**Figure 3.2**). Overall, the risk of bias for each study were low, however, three studies have small samples size (<100).

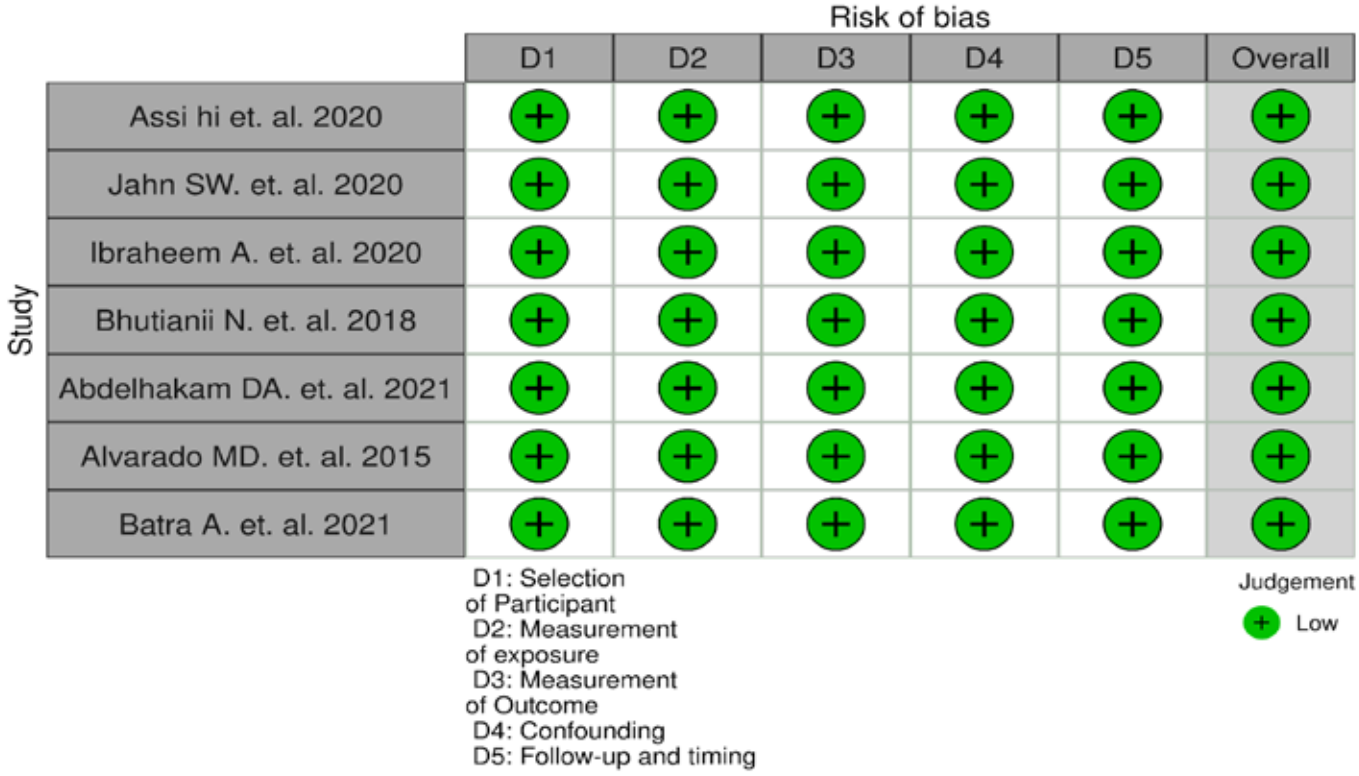


Figure 3.2: Summary of risk of bias assessment for cohort study using CASP checklist

Studies populations of the included studies

Population studies in the SR by Villareal-Garza C. et. al. (2020) were young women with early-stage breast cancer. However, the authors used certain threshold to define young age as it was varied widely in the included studies. The age cut-off ranged from <35 to ≤55 years[20]. Table 2 showed number and various ranged of 'young' patients in the included studies.

Table 2: Proportion of young patients participating in genomic risk trials stratified according to how 'young age' was defined

Definition of young used	Total # of participants	# of young patients (%)
<35 years	7,559	134 (1.8%)
≤35 years	471	22 (4.7%)
<40 years	444,070	14,946 (3.4%)
≤40 years	311,088	13,233 (4.3%)
<45 years	7,444	1,238 (16.6%)
<50 years	117,223	25,242 (21.5%)
≤50 years	13,111	4,431 (33.8%)
<55 years	142	67 (47.2%)
≤55 years	936	459 (49.0%)
Premenopausal	3,065	1,218 (39.7%)

*Table was adopted from Table 1 of Villareal-Garza C. et. al. (2020)[20]

Population of the included studies in SR by Blok EJ et. al. also varied among patients with early-stage breast cancer (LN- / LN+ / combination of LN- LN+ / ER+ / HER2-ve). The age of patients ranged from 35 to 70 years old.[21] As for SR by Scope EJ. et. al. The youngest patient from one of the included studies was 23 years old and the eldest was 89 years old (ER+ / LN- / HER2+ / HER2- / PR+ / LN+ / LN-/LN1-3 / LN1).[22]

For the cohort studies, the age of population studied ranged between 31 and 81 years old. All of them had early-stage breast cancer (HR+ / HER2- / LN+ / LN0 / LN1-3 / N+ / N0) and either had stage I, II and III or tumour size of less than 2 cm or ≥ 2cm. On the other hand, only one study emphasized results based on menopausal status of the patients.[23-29]

5.2.3 Efficacy / Effectiveness

Overall results of molecular profiling assays

There were three SR and one cohort study reported the overall result of molecular profiling assays, regardless the individual assays results. An SR by Villareal-Garza C. et. al. reported that by age, larger proportion of high genomic risk tumour were observed in women ≤40 years

compared to older patients, this observation was significant in Oncotype DX (p<0.001), MammaPrint (p<0.001) and EndoPredict (p=0.042). MammaPrint and EndoPredict classified two third of tumour in patients ≤40 years as high-genomic risk compared to half in older patients. The SR also found that with molecular profiling assays, high testing probability was among younger patients (≤40 years) compared to older patients (≥40 years) which was 32% versus 29%, p = 0.033.[20] Another SR by Blok EJ. et. al. showed that tumours with a combination of grade 1, PR+ and/or have a Ki67 expression score lower than 10% were always stratified by molecular profiling assay as low-risk. Meanwhile, tumours with a combination of grade 3, PR-ve and/or have a Ki67 score more than 40% were almost always high-risk with molecular profiling assays.[21] The SR by Blok EJ. et. al. evaluated 28 studies regarding a clinical utility of the molecular profiling assays, regardless the assays types. In general, the SR reported that de-escalation from chemotherapy to no therapy or endocrine therapy alone was higher than the escalation towards chemotherapy, which led to a decrease in chemotherapy use for all molecular profiling assays.[21]

Individualise results of molecular profiling assays

a. Oncotype DX

An SR by Villareal-Garza C. et. al. in 2020 evaluated the molecular profiling assays specifically in young women with breast cancer. The SR included 71 studies with a total number of 561,188 patients. Almost all the patients underwent Oncotype DX test (96.34%), MammaPrint (3.32%), EndoPredict (0.24%) and others (0.10%). Nine out of 71 studies assessed prognostic value of genomic signatures in young women with breast cancer. The prognostic performance of Oncotype DX in N0 patients showed that, the six years' distant recurrence free survival (DRFS) were 94.4% in low-, 96.9% in intermediate-and 85.1% in high-genomic risk (p < 0.001). The proportion of patients that received chemotherapy for each risk category was 21.2% in low-genomic risk, 44.1% in intermediate-genomic risk and 91.7% in high-genomic risk. For patient with N1 disease, in those who were treated with chemotherapy, six years DRFS rate were 92.3% in low-, 85.2% in intermediate- and 71.3% in high-genomic risk. A multivariate analysis showed that tumour size, node status, histological grade and chemotherapy used in high-risk RS were associated with the risks of distant recurrence ($HR_{\text{recurrence score } \leq 25 \text{ vs } >25}$ 0.31; p = 0.01).

In women with stage I-II, HR+/HER2-ve, N0 disease, a low- to intermediate-risk group had excellent five-year overall survival (OS) despite of low chemotherapy used with no differences

in risk category (99%; p = 0.93). Meanwhile in high-risk group, the 5-year OS was significantly lower (94% for those with a recurrence score (RS) of 26 – 30 and 92% for RS > 30) even though majority of the patient's received chemotherapy with estimated $HR_{high\ vs\ low\ risk}$ of 5.13, p < 0.001). One study reported that patients <40-years old with high RS had similar disease-free survival (DFS) as older counterpart when treated with chemotherapy. On the other hand, one study reported that patients ≤40-years old who had high-intermediate risk scores (16-25) were not benefited in DFS with an additional chemotherapy in their treatment.[20]

The SR by Villareal-Garza C et. al. included five studies regarding the use of chemotherapy based on Oncotype DX risk stratification. The included studies reported on young women with HR+/HER2-ve, LN+ or stage I-II, HR+/HER2-ve, N0 who were either low-risk or intermediate-risk and had high histological grade and large tumour who received more chemotherapy compared to elderly of the same risk category. One of the studies reported that approximately 43% of the young women with low-RS (RS < 11) received chemo compared with 28% of the elderly of the same risk category (p = 0.03). Another study reported that 38% of intermediate-RS (RS = 11-25) young women with large tumour and high clinical stage underwent chemotherapy compared to 15% in elderly of the same risk category.[20]

Another SR by Blok EJ et. al. evaluated whether molecular profiling assays result can be predicted by standard clinicopathological parameters. There were 12 studies included consisted of 11 studies on Oncotype DX and only one study on MammaPrint. Further assessment on the Oncotype DX found that in LN- patients, the distant recurrence free survival (DRFS) outcome between low-, intermediate- and high-risk were statistically significant. Besides, there was statistically different effect of chemotherapy in those three risk groups with a significant interaction between chemotherapy and Recurrence score (RS). In LN+ patients there were also significant interaction between RS and clinical benefit of chemo for the first five years after treatment. Blok EJ et. al. also reported the TAILORx trial results of clinical used of Oncotype DX in chemotherapy decision. The trial involved patients with ER-ve and/or PR+, N-breast cancer and were already assigned with chemotherapy based on NCCN-guidelines. However, based on the RS the low-risk patients received endocrine therapy only, high-risk patients received both endocrine and chemotherapy, and for intermediate-risk the treatment allocation was either endocrine therapy alone or combination of endocrine and chemotherapy. For this trial, only the low-risk results were published with involvement of 1,626 patients who

received endocrine therapy. The results showed that the DFS rate at five-years was 93.8% (95% CI 92.4% - 94.9%), DRFS was 99.3% (95% CI 98.7% - 99.6%) and rate of OS was 98% (95% CI 97.1% - 98.6%). This finding showed that the genomic testing able to identify patients with a good prognosis without chemotherapy despite a clinical indication for chemotherapy. However, out of 1,626 patients, 6 patients received adjuvant chemotherapy as one of them had recurrence despite adjuvant chemotherapy. Another trial reported by Blok EJ et. al. was WSG PlanB trial which involved 3,198 clinically high-risk patients and 41.1% of them were N+ breast cancer. Initially this trial was designed to compare two chemotherapy regimens, however, later the researcher's omitted chemotherapy in patients with low-risk Oncotype DX test result, despite their high clinical risk. There were 348 patients who were actually clinical-high risk and their chemotherapy were cancelled as their RS less than 12 (low-risk). After three years followed-up, the DFS for those 348 patients were 98.4% which indicated that the genomic subtyping with the Oncotype DX able to identify clinically high-risk subgroup with an excellent prognosis without chemotherapy.[21]

The SR by Blok EJ. et. al. evaluated 28 studies regarding a clinical utility of the molecular profiling assays. Based on the included studies, the decrease in chemotherapy was more pronounced for Oncotype DX (45.7% from chemotherapy to endocrine therapy alone or no adjuvant therapy) compared to MammaPrint (32.2% decreased). Besides, there was one study reported no difference in the use of chemotherapy observed despite an increase of Oncotype DX used from 9% to 17.2% between 2008 to 2011. Two studies reported an increased in used of chemotherapy after genomic test from 26% to 22%. One study in N+ population reported that after Oncotype DX test the used of chemotherapy decreased from 70% to 24.5%. Meanwhile for MammaPrint, one study reported that 10% lower rate of chemotherapy for patients with genomic testing.[21]

An SR by Scope A et. al. evaluated the clinical effectiveness of molecular profiling assays and expanded immunohistochemistry (IHC) test to guide the use of chemotherapy in early breast cancer. The SR included 41 studies that were published between 2002 and 2016 involving women with early invasive breast cancer. Out of 41 studies, 32 studies were on Oncotype DX, six studies were on MammaPrint and the other two studies on other types of molecular profiling assays. For Oncotype DX, four studies were related to the prediction of treatment effect with adjuvant chemotherapy. In ER+, LN- patients, one study found that the RS was correlated with

chemotherapy benefit which was 10-years DRFS with significant increased benefit in high-risk group compared to low-RS group. In ER+, LN+ patients another study also reported that the RS alone remained the best predictor of chemotherapy benefit. The RS was also found to be a good prognostic measure for tamoxifen-treated patients with positive nodes and predicted significant benefit of chemotherapy in tumours with high-RS. Another 28 studies reported on the treatment decision based on the Recurrence Score. Overall, the Oncotype DX led to the changes of treatment recommendations between 21% to 74%. The changes from chemotherapy to no chemotherapy ranged from 6% to 51% and most of the changes were de-escalation of the chemotherapy recommendation. Only one study reported increased in chemo therapy after the genomic test.[22]

A retrospective cohort by Assi HI et. al. was conducted to determine the impact of molecular profiling assays on physicians' treatment decisions and the percentage of patients de-escalated or escalated the treatment. The study involved only 75 early-breast cancer patients ($T_{1\text{-}2}\,N_0$, included T1pN1mic) HR+/HER2-ve within range of age 31 to 81 years old. Out of 75 patients, 84.93% were low-grade tumours and 15.06% were high-grade tumours. Fifty patients (66.67%) underwent Oncotype DX test (21 patients with low-RS, 26 patients with intermediate-RS and 3 patients with high-RS), 14 patients (18.67%) underwent EndoPredict test (low-score in 10 patients and high-score in four patients) and 11 patients (14.67%) underwent Prosigna (PAM50) test (low-score in three patients, intermediate-score in seven patients and high-score in one patients). Before the molecular profiling assays was performed, the physicians already planned for treatment in each patient either to receive endocrine therapy alone or to receive both endocrine and chemotherapy. Ten patients were planned to receive endocrine therapy alone, however, after the molecular profiling assays, seven patients required both endocrine and chemotherapy. In 44 patients who were planned to receive chemotherapy and endocrine therapy before the assays, 19 of them (43.2%) were deescalated to endocrine therapy only after the test. Out of the 75 patients, 21 patients were not planned for any treatment. After the molecular profiling assays, the physician decided to proceed with endocrine therapy alone in 13 patients and the rest with both endocrine and chemotherapy. The overall results were summarised in Table 3. The authors also further analysed the treatment decision based on the RS of the Oncotype DX. From the assessments, 17 out of 21 patients who were classified as low-RS received endocrine therapy alone and the other four patients received chemotherapy in addition to initial planned of endocrine therapy. In intermediate-RS, 19 patients received both

endocrine and chemotherapy and all patients in high-RS received chemotherapy. Further analysis of correlation assessment between the RS and tumour grade showed that most of the patients with high-grade tumours had either intermediate or high-RS value ($p < 0.001$). Another correlation was between RS and Ki-67 which showed that Ki-67 was significantly associated with RS categories ($p < 0.05$); Ki-67 < 14 associated with low or intermediate RS and Ki-67 > 14 associated with high-RS.[23]

Table 3: Pre- and Post-Genomic Test Plan

Pre-genomic test plan	Post-genomic test plan	
	Endocrine therapy (N = 35)	**Endocrine + chemotherapy (N = 40)**
Endocrine therapy (N = 10)	3 (stick on pre-test planned)	7
Chemotherapy + Endocrine therapy (N = 44)	19	25 (stick on pre-test planned)
Undecided therapy (N = 21)	13	8

*Adapted from Figure 2 Assi HI et. al. (2020)[23]

b. MammaPrint

For MammaPrint, the SR by Villareal-Garza C et. al. reported the results of MINDACT phase III trial that evaluated the prognostic performance in young patients which also involved reclassification of risk category by MammaPrint. The study involved 2,226 of patients <50-years old and 122 patients <35-years old. From the post-hoc analysis of the trial, MammaPrint reduced the proportion of high-risk in patients aged <45 years who were initially being classified as high-risk by clinical parameters-only assessment; 48% versus 61%, respectively. The trial also reported that, sub-classification of women <45 years in clinical-parameters high-risk category to low-risk category by MammaPrint was translated into 5-year DMFS of 95.5% compared to the high-risk category by MammaPrint (89.2%). Meanwhile in clinical-parameters low-risk category, either reclassified as low-risk or high-risk by MammaPrint, the prognosis was good as the DMFS rate were 98.3% and 97.4%, respectively. Subsequent analysis reported that, the outcome of clinical parameters-only high-risk patients ≤50 (already reclassified into low-risk by MammaPrint) who were treated with endocrine alone was not significantly worsen compared to those who received chemotherapy (DMFS absolute difference of 3% at 5 years in women aged ≤ 50 years versus 0.2% in older patients).[20]

The SR by Blok EJ et. al. reported that LN- patients had significant prognostic value with MammaPrint. As for LN+ patients the hazard ratios (HRs) for DMFS and breast cancer specific

survival (BCSS) showed a significant difference in prognosis for low-risk score compared to high-risk score.[21]

The SR by Blok EJ et. al. reported the results from MINDACT trial that evaluated the used of MammaPrint together with Adjuvant Online! in order to assess the used of MammaPrint in chemotherapy decision. The results were divided based on two patients' risk subgroups. Subgroup one was on patients who were initially classified by clinical assessment as high-risk (clinically high-risk) but reclassified as low-risk by MammaPrint (MammaPrint-low risk) and were randomly allocated to receive no chemotherapy; first finding showed that the DMFS of this subgroup was 94.7% at five-years which was significantly higher compared to a pre-determined null hypothesis of 92%. This finding indicated that the prognosis of the MammaPrint-low risk patients without chemotherapy was good enough to justify the abstention of the chemotherapy. Second finding was compared between the clinically high-risk patients and the MammaPrint-low risk patients with and without chemotherapy, the HR was 0.65 which favoured towards chemotherapy and was significant for DFS (90.3% versus 93.3%; p = 0.026) but not for DMFS (94.7% versus 96.7%; p = 0.106) or overall survival (OS) (97.2% versus 98.8%; p = 0.245). This finding actually indicated that although the prognosis of the clinically high-risk group was good without chemotherapy, it was significantly better with chemotherapy. Subgroup two was clinically low-risk patients who were reclassified as MammaPrint high-risk, the finding showed that there was no statistically significant benefit of chemotherapy was observed for either DMFS (HR 0.90, 95% CI 0.40 – 2.01), DFS (HR 0.74, 95% CI 0.40 – 1.39) or OS (HR 0.72, 95% CI 0.23 – 2.24) which indicated that high-risk MammaPrint test result did not predict the effect of chemotherapy for these group.[21]

The SR by Scope A et. al. included six studies that used MammaPrint in addition to clinicopathological factors that led to change in treatment recommendations. The changes were between 18% and 40% of all tested patients and between 2% and 32% were recommended to change from chemotherapy to no chemotherapy. One of the studies reported that 48% patients were recommended for adjuvant treatment based on CBO guideline 2004 alone, however, after MammaPrint test was introduced, the percentage was increased to 62%.[22]

c. EndoPredict

For EndoPredict, the SR by Villareal-Garza C et. al. reported on GEICAM 9906 trial which concerned on pre-menopausal women (300 [54%] out of 555 patients with HR+/HER2-ve, LN+), reported that the DMFS at 10-years in the pre-menopausal women was 93% in low-risk score group compared to 67% in high-risk score group ($p<0.0001$).[20]

Villareal-Garza C. et. al. examined the used of chemotherapy according to stratification by EndoPredict and found that 1/11 (9%) young women ≤40 years and 4/35 (11%) of older premenopausal women were recommended for chemotherapy by the institutional tumour board despite of having low EPclin risk score.[20]

The SR by Blok EJ et. al. reported that EndoPredict differences between high- and low-risk were associated with low proportion of distant metastases in low-risk group.[21]

d. Prosigna/PAM50

Blok EJ et. al. reported that the included studies showed a good discrimination and significant interaction between treatment and outcome. There were also studies showed a significant association with distant recurrences.[21]

Concordance and correlation between molecular profiling assays

There was no prospective study comparing between assays retrieved. Only few studies that looked at the association, concordance and correlation between assays were included in this review.

a. Oncotype Dx versus MammaPrint

Ibraheem A. et. al. conducted a study to compare the prognostic performance of the two commonly used molecular profiling assays; Oncotype DX and MammaPrint in patients with HR+ breast cancer. The study included 144,357 patients who received Oncotype DX and 5,047 patients who received MammaPrint. The study reported that MammaPrint was mostly ordered among patients with high-risk clinical-pathological inclusive lymph node positivity, larger tumour size, higher tumour grade and lymphovascular invasion. The percentage of high-risk were 46.0% in MammaPrint and 37.2% in Oncotype DX. Propensity score matching was performed to ensure that patients who received Oncotype DX and MammaPrint were comparable. The propensity score showed that 5,042 patients who actually received MammaPrint were matched in 5,042 patients who actually received Oncotype DX. The matched cohort was followed with

a median of 33 months with interquartile range of 21 to 49 months. In the matched cohort of 5,042 patients with MammaPrint, 2,908 had genomic low-risk and 11.5% of them received chemotherapy. Meanwhile another 2,134 patients had genomic high-risk and 80.0% of them received chemotherapy. As for matched cohort of Oncotype DX, 1,104 patients had low-risk RS where 5.8% of them received chemotherapy and 19.9% of 3,068 patients with intermediate-risk RS received chemotherapy. Meanwhile in 834 patients who had high-risk RS, 73.7% of them received chemotherapy. The overall survival analysis also conducted and the analysis showed that 5-year risk of dying among high-risk patients of MammaPrint were 9.3% and 12.4% in Oncotype DX. Multivariable Cox models reported that separation between recurrence risk groups (low- and high-risk) with regard to survival was similar between both assays (range of C-index between the risk-groups 0.5 – 0.6), although the prognostic value was slightly higher for MammaPrint.[24]

Another study by Bhutianii N et. al. evaluated the molecular profiling assays used over time and the effect of the tests on administration of postoperative chemotherapy. The test involved were MammaPrint and Oncotype DX. The Oncotype DX was the most common test used throughout the study period; 94.9% to 92.7% of all molecular profiling assays issued from 2011 to 2014 and the used was more common among patients with stage I diseases. As for MammaPrint, the used was increased over time; 2.3% to 4.7% of all molecular profiling assays used from 2011 to 2014; $p = 0.03$ and the used was more common in patient with stage II and stage III inclusive ER-ve, PR-ve, HER2+ and patients with LN+ tumours. The used of molecular profiling assays also associated with a decrease rate of chemotherapy administration (24.5% versus 37.2% without molecular profiling test; $p < 0.001$). By types of molecular profiling assays, a post-operative chemotherapy was highly administered among patients who had MammaPrint test compared to Oncotype DX (41.3% MammaPrint versus 23.4% Oncotype DX; $p < 0.001$) which was persisted among stage II and stage III patients. The study also conducted subgroup analysis to observe post-operative chemotherapy administration by the risk score, overall, the chemotherapy was administered more in MammaPrint group as shown in Table 4.[25]

Table 4: Post-operative chemo administration by recurrence risk (MammaPrint versus Oncotype DX)

Recurrence Score (RS) threshold		Oncotype DX	MammaPrint
Low-risk	RS < 11	4.4%; p	11.3%; p < 0.01 compared to Oncotype DX regardless of the threshold
	RS< 18	6.5%	
High-risk	RS ≥ 26	53.3%	88.7%; p < 0.001 compared to Oncotype DX regardless of the threshold
	RS ≥ 31	57.1%	

An SR by Blok EJ et al in 2018 included 149 papers to evaluate four molecular profiling which were Oncotype DX, MammaPrint, Prosigna/PAM50 and EndoPredict. The SR reported on a few studies that directly compared test results of multiple tests performed on one tumour. In one of the trials, three molecular profiling tests were performed in similar cohort, the result showed that MammaPrint classified the highest percentage (38.6%) of high-risk patients group compared to Prosigna (34.5%) and Oncotype DX (17.9%).[21]

Blok EJ et. al. also reported on the studies that compared chemotherapy treatment decision of the same patients before and after the molecular profiling test. Although the SR reported that the overall decrement in chemotherapy was the most pronounced in Oncotype DX compared to MammaPrint, the results should be interpreted accordingly because of large difference in the number of studies per test, the baseline patients' characteristics and the study designs. For Oncotype DX, although there were studies observed a decreased in chemotherapy used during designated years and increased in molecular profiling test, no direct relation was analysed. On the other hand, one study reported of no difference in the used of chemotherapy despite an increase of Oncotype DX used from 9% to 17.2%. However, one study showed that used of chemotherapy was significantly increased in patients who underwent Oncotype DX test compared to patients who did not; 26% versus 22% (p < 0.01), respectively. The study also reported that within seven to eight years, the used of Oncotype DX was increased from 8% to over 25%, while the percent of women receiving chemotherapy decreased modestly from 26% to 22%.[36] However, in another study of N+ patients, comparing Oncotype DX group and no Oncotype DX (control group), 24.5% chemotherapy was used after the test compared to 70% without Oncotype DX test.[37] Study involved MammaPrint also showed that chemotherapy used was 10% lower in patients who had MammaPrint test compared to none.[21]

b. Oncotype DX versus Prosigna

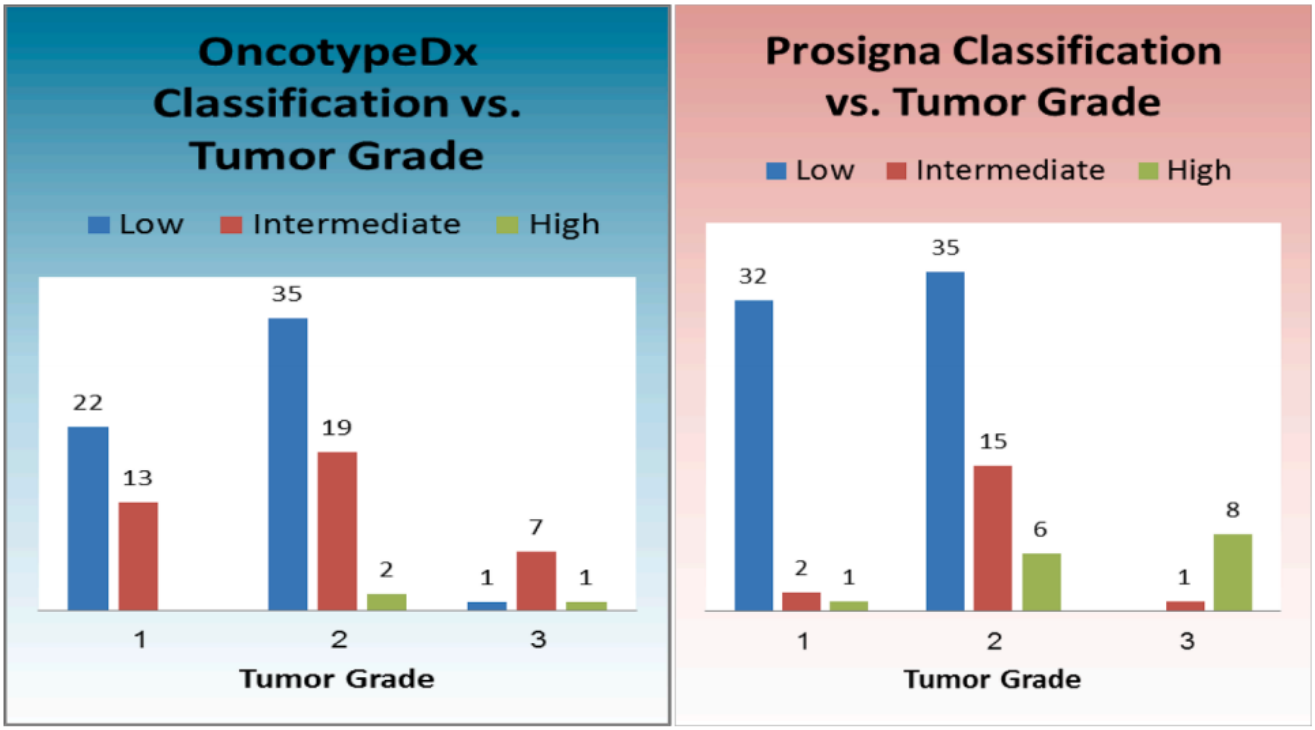
One study in SR by Blok EJ et. al. reported that based on Spearman correlation coefficient, Oncotype DX and Prosigna had very week positive correlation (r_s = 0.08) as 57.1% patients who were classified as high-risk on Prosigna were actually low-risk by Oncotype DX.[21]

Abdelhakam DA. et. al. conducted a retrospective cohort study to assess the agreement between Oncotype DX and Prosigna and compared each recurrence risk scores with commercial cut-off points. The commercial cut-off point used to compare between those two molecular profiling was Ki-67 which was determined with IHC of FFPE sections where it's determined the percentage of nuclei with positive staining in the tumour cells, the Ki-67 prognostic cut-offs point were <14% (low-risk score), 14 – 20% (intermediate-risk score) and >20% (high-risk score). The study involved 100 samples of breast cancer patients with average age of 62.4 years with more than 90% of them were >50-years old. When referring to menopausal status, 80% of the patients were post-menopausal women and 20% were pre-menopausal women. All the included patients underwent Oncotype DX test and the same sample were retested with Prosigna. Table 5 below showed a distribution of patients classified into risk groups based on score by Oncotype Dx and Prosigna risk score. According to the Oncotype DX test, 57 patients were categorised as low RS versus 67 patients by Prosigna. As showed in Table 5, 43 cases were agreed by both tests as low-risk (43/57; 75.4%) and the remaining cases were categorised as intermediate risk (8 cases; 14%) and high-risk (6 cases; 10.5%) by Prosigna. Meanwhile in intermediate RS, only eight cases out of 39 cases (20.5%) were similarly categorised by Prosigna and another 31 cases were categorised differently by Prosigna. For high-risk cases, Oncotype DX classified four cases as high-RS and only 1 case (25%) agreed by Prosigna and the other three cases were categorised low-risk (2 cases; 50%) and intermediate risk (1 case; 25%) by Prosigna. Based on this finding, the overall agreement between Oncotype DX and Prosigna was 52% (43 in low-risk, 8 in intermediate-risk and 1 in high-risk). The one-step disagreement between Oncotype DX and Prosigna either low- to intermediate-risk or intermediate- to high-risk was 40%. When compared with Ki-67 scores, 31 out of 40 cases showed an agreement between Prosigna ROR score and Ki-67 score. Meanwhile, six cases showed an agreement between Oncotype DX RS and Ki-67 scores. Meanwhile, another 8% was two-step disagreement which was either high- to low-risk or vice versa. Out of the 8 cases of two-step disagreement, 7 cases were agreement between Ki-67 and Prosigna. Based on Spearman's correlation analysis, it showed that Oncotype DX and

Prosigna had poor correlation; r_s = 0.195 p (2-tailed) = 0.052. On the other hand, although weak correlation was observed in both test and in post-menopausal women, the correlation was significant; r_s = 0.276, p = 0.013. Correlation between Ki-67 and both tests showed that Prosigna correlated very well with Ki-67 expression; r_s = 0.797, p (2-tailed) = 0.000 but very weak correlation with Oncotype DX; r_s = 0.136, p (2-tailed) = 0.177). The authors also assessed three recurrence cases in the samples. Further observations on the three recurrence cases found high ROR score in all three cases but low RS in 2 cases and intermediate RS in one case. The Ki-67 score also high in those three cases that matched with Prosigna ROR scores. Then, observation on tumour grade showed that Prosigna recurrence scores correlated better with tumour grades (1, 2,3); r_s = 0.595, p = 0.0000) compared to Oncotype DX; r_s = 0.142, p = 0.158. The details of the results were in Figure 4a. Besides that, the distribution of cases with regard to intrinsic subtypes (luminal A and luminal B) also observed and the results in Figure 4b.[26]

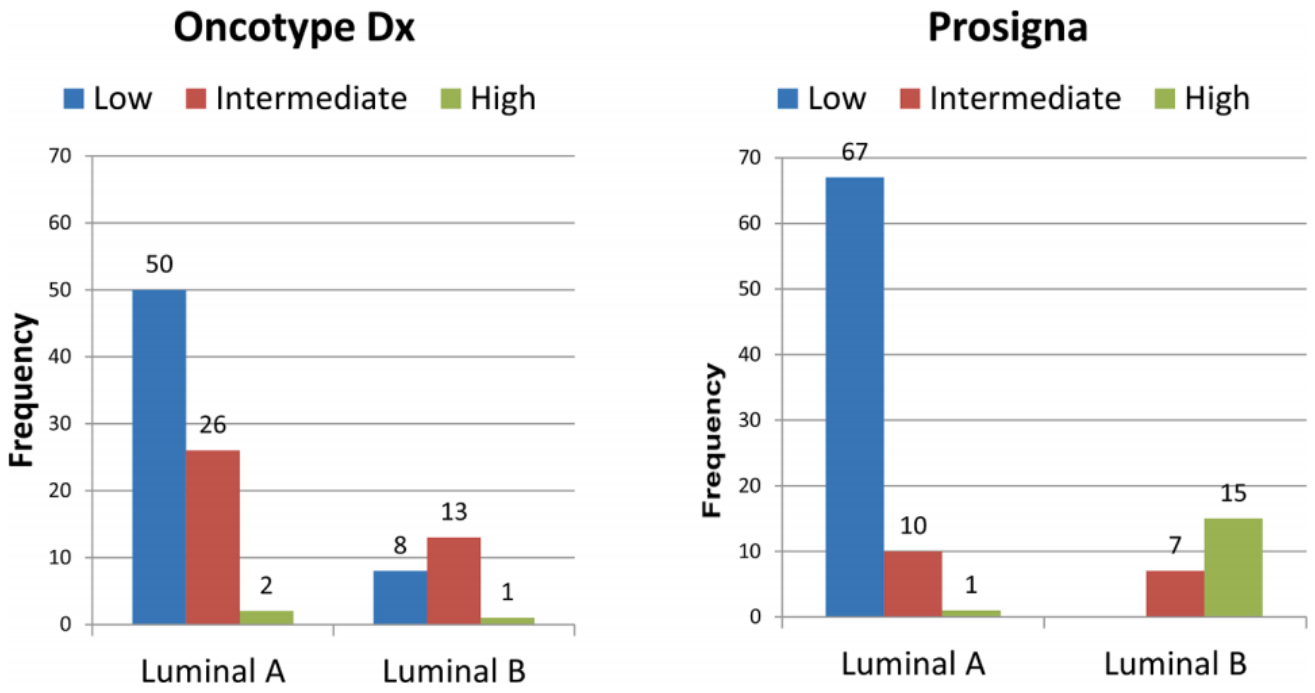
Table 5: Molecular Profiling score based on Oncotype DX and Prosigna

Prosigna risk groups	Oncotype DX Recurrence score			Total
	Low (<18)	Intermediate (18 – 30)	High (>31)	
Low (<40)	43	22	2	67
Intermediate (40-60)	8	8	1	17
High (61-100)	6	9	1	16
Total	57	39	4	**100**



* Figure adopted from Figure 3 of Abdelhakam et. al.[26]

Figure 4a: Distribution of cases Oncotype DX RS and Prosigna Score in relation to tumour grade



* Figure adopted from Figure 3 of Abdelhakam et. al.[26]

Figure 4b: Distribution of Oncotype DX RS and Prosigna Score within luminal A and luminal B

Another study by Alvarado MD et. al. also assessed the agreement between Oncotype DX and Prosigna. Fifty-two (52) patients were finally included which involved majority of invasive ductal carcinoma (73.1%), tumour size ≤ 2cm (78.9%) and grade 1 or 2 tumour (90.4%). More than half of the patients (55.8%) were ≥ 70 years old. Overall, the distribution of the RS and ROR scores showed a marked differences between both assays as more patients were classified as low-risk and fewer patients were classified as intermediate or high-risk by the RS results compared to Prosigna. Table 6 showed the risk stratification score by both tests. The agreement between both tests were observed and overall agreement for risk classification based on RS and the Prosigna score results was 53.8%, details of the results were in Table 7. From the table it showed that 37 patients with low RS, only 22 was classified as low-risk in Prosigna and the rest was intermediate-risk (11 patients) and high-risk (four patients). Based on the above findings, further Spearman's correlation analysis was conducted and it showed that correlation between RS and Prosigna score was very weak (r_s = 0.08; 95% CI -0.2 – 0.35). The authors also evaluated quantitative ER expression by RT-PCR. The result showed a wide range of expression within each Prosigna score risk group. Especially in all four patients who were classified as high-risk by Prosigna but low RS exhibited high ER expression. In addition, two patients with ER expression close to positivity threshold and high RS were classified as low or intermediate by Prosigna. This study also evaluated the intrinsic subtypes (luminal A and luminal B) distribution between Oncotype DX RS and Prosigna score, the distribution was shown in figure 5. The figure showed that among 38 patients (38/52; 73.1%) who were classified as luminal A by Prosigna score were in either low- or intermediate-risk category. However, in the same intrinsic group, only one patient (2.6%) was characterised as high-risk by Oncotype DX. As for 12 patients (23.1%) who were classified as luminal B by Prosigna score, 10 of them had low RS but was classified as intermediate-risk and high-risk by Prosigna.[27]

Table 6: Distribution of Recurrence Score and Prosigna Results

Risk-group	Oncotype DX RS (Median RS 12 [range 0 – 36])	Prosigna ROR score (Median RS 39 [range 0 – 88])
Low-risk	37 patients (71.2%)	28 (53.8%)
Intermediate-risk	12 patients (23.1%)	17 (32.7%)
High-risk	3 patients (5.8%)	7 (13.5%)

Table 7: Agreement in risk group assignment between Recurrence Score and Prosigna results in post-menopausal, node-negative, ER-positive patients (N = 52)

Prosigna risk groups	Oncotype DX Recurrence score			Total
	Low < 18	Intermediate (18 – 30)	High ≥ 31	
Low < 40	22 (79%, 95% CI 59 – 92%)	5 (18%, 95% CI 6 – 37%)	1 (4%, 95% CI 0 – 18%)	28
Intermediate (41 – 60)	11 (65%, 95% CI 38 – 86%)	5 (29%, 95% CI 10 – 56%)	1 (6%, 95% CI 0 – 29%)	17
High (61 – 100)	4 (57%, 95% CI 18 – 90%)	2 (29%, 95% CI 4 – 71%)	1 (14%, 95% CI 0 – 56)	7
Total	37	12	3	**52**

*Table adopted from Alvarado MD et. al.[27]

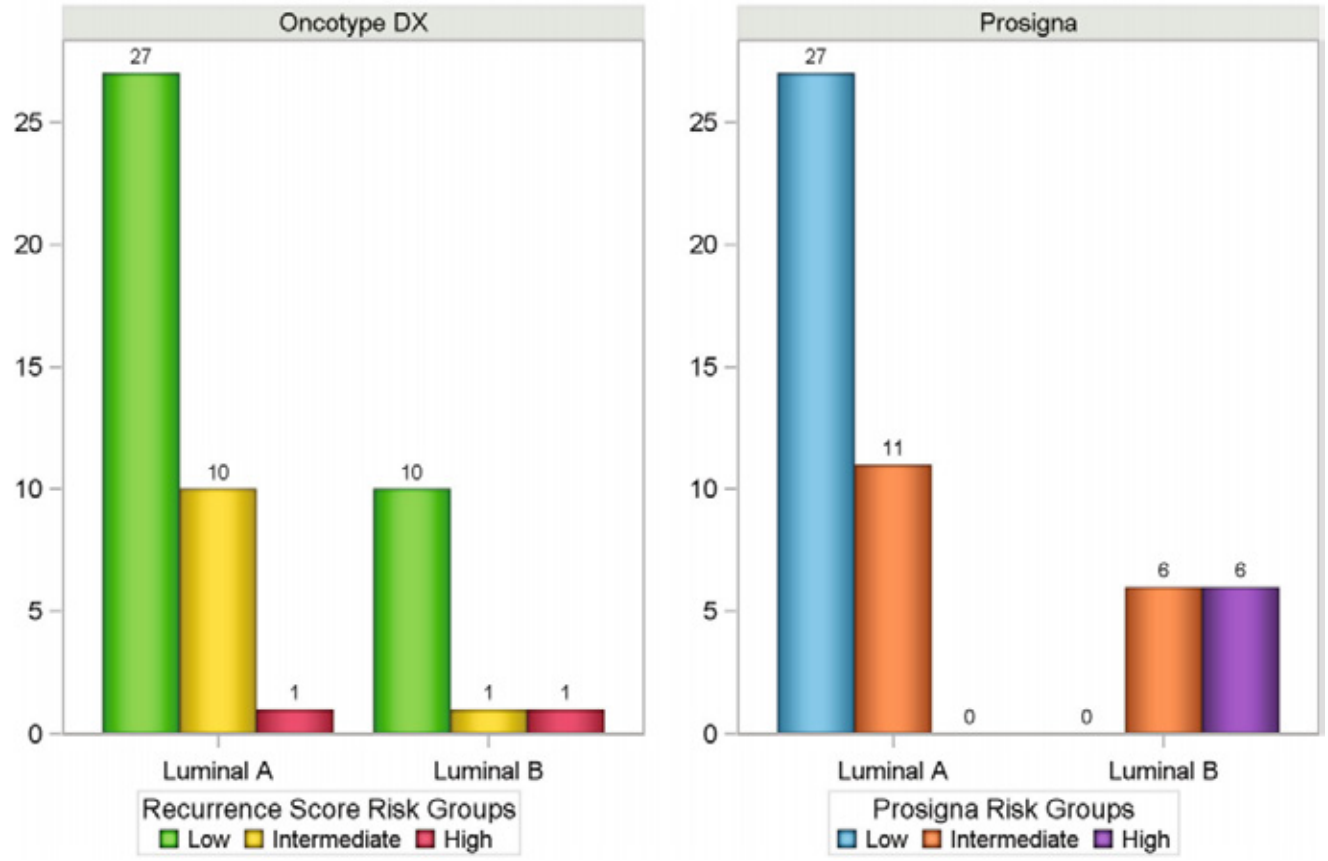


*Figure were adopted from Alvarado MD et. al.[27]

Figure 5: Distribution of Recurrence Score and Prosigna Score within luminal A and luminal B subtypes

c. MammaPrint versus EndoPredict

Jahn SW et. al. conducted retrospective cohort to compare concordance between MammaPrint and EndoPredict. The study involved 94 patients with ER+, HER2-ve, tumour-node-metastases (TNM) stage I and II breast cancer below 5 cm in diameter with up to three positive lymph nodes. Out of 94 cases, 79.8% of cases were high-risk by EPscore, 44.7% of high-risk with EPclin and 42.6% high-risk by MammaPrint. According to histopathological Ki-67 index, there

was significant association between the Ki-67 index with EPclin and MammaPrint, however, histopathological T-stage (pT), nodal status (pN) and clinical risk stratification as per criteria in MINDACT trial correlated with EPclin but not with MammaPrint (Table 8). The study reported that case per case MammaPrint to EPclin risk predictions were discordant in 36%. The study also showed significant association between MammaPrint and EPclin (p=0.01) with fair agreement between both assays; κ = 0.27, 95% CI 0.069 – 0.46. However, an observation in 43 clinically high-risk cases, the molecular profiling assays resulted in 93% of high-risk by EPscore, 76.7% by EPclin and 46.5% by MammaPrint. Based on this results, the discordant risk predictions now increased to 44% and MammaPrint to EPclin results failed to show a significant association (p = 0.294, κ = 0.15, 95% CI -0.089 – 0.39). The clinically high-risk cases were 65% significantly classified as high-risk in EPclin than MammaPrint (p = 0.004).[28]

Table 8: Association of MammaPrint and EndoPredict with clinical variables (Chi-Square/Fisher Exact)

Molecular profiling test/ Clinical variables	pT	pN	Grade	Ki-67	Progesterone receptor	MINDACT
MammaPrint	0.564	0.108	0.017	0.001	0.488	0.476
EPclin	< 0.001	< 0.001	0.258	0.003	0.073	< 0.001
EPscore	0.166	0.795	0.011	0.207	0.681	0.003

*Adopted from Supplemental table 2 Jahn SW et. al. (2020)[28]

Molecular profiling assays versus clinicopathological model

Batra A. et. al. in 2021 conducted a retrospective cohort to compare the characteristics of patients who underwent Oncotype DX and Prosigna test. The authors also determined the utility of the clinical-pathologic features to predict the genomic-risk of the recurrence through development of simple clinical-pathological mode (CP model). The study involved 366 patients who were diagnosed with HR+/HER-ve, N- and already undergone Oncotype DX and Prosigna test from October 2017 to March 2019. Out of 366 patients, 135 (36.9%) were tested with Oncotype DX and another 63.1% (231) patients were tested with Prosigna. According to the molecular profiling assays test, 64 patients (17.5%) were categorised as high-risk and 302 patients (82.5%) as low-risk. The risk was also sub-classified according to age, tumour size and grade as well as by expression of progesterone receptor (PR). The sub-classification showed that older patients, larger tumour size, high-grade tumour and lower expression of PR were higher in high-genomic risk group than low-genomic risk (Table 9). In addition to that, multivariable analyses were conducted and the results showed that patient with larger tumour size (>20mm), Allred PR expression of 0-4 and higher-grade tumour (grade III) had higher

likelihood ratio (LR) of high-genomic risk; odds ratio 3.84, 95% CI 1.84 – 6.98 (p < 0.001), odds ratio 3.46; 95% CI 1.76 – 6.82 (p < 0.001) and odds ratio 7.24; 95% CI 3.82- 13.70 (p < 0.001), respectively. Based on this, the authors constructed CP model to predict the LR of the genomic risk group. The model rounded the coefficient of the regression analysis with score range between 0 – 4 where the minimum CP risk score (0) would be attributed to a patient with grade I/II, tumour size ≤ 20mm and PR expression of 5 to 8 categories. Meanwhile the maximum risk score was four for patients with tumour size > 20mm, grade III and low PR expression. The authors later assessed the concordance of the CP model score with the molecular profiling assays groups and patients' age, the results were shown in Table 10 and Table 11. Sensitivity analysis for clinical-pathologic risk score cut-points of 0, ≥ 1, ≥ 2 and ≥ 3 was performed to predict genomic low risk category. The analysis showed that the specificity was the highest with cut-point of 0 (98.4%) with sensitivity of 55.9%, PPV of 99.45 and NPV of 32.1%), other results in Table 12.[29]

Table 9: Clinical-pathological factors and genomic-risk score

Clinical-pathological factors	Low-risk	High-risk	p-value
Age > 50 years old	8.9%	84.4%	0.013
Tumour size > 20mm	17.9%	43.8%	< 0.001
Tumour grade III (high-grade)	22.8%	70.3%	< 0.001
PR expression (lower expression)	14.9%	42.2%	< 0.001
Others: histological subtype, lymphovascular invasion, facility of tumours and HER2 expression	No difference		

Table 10: Distribution of Clinical-Pathologic score and genomic risk category by GEP type

CP model score	All patients (n = 366)		Oncotype DX (n = 135)		Prosigna (n = 231)	
	Low-risk	High-risk	Low-risk	High-risk	Low-risk	High-risk
0	169 (99.4%)	1 (0.6%)	58 (98.3%)	1 (1.7%)	111 (100.0%)	0 (0.0%)
1	59 (78.7%)	16 (21.3%)	20 (83.3%)	4 (16.7%)	39 (76.5%)	12 (23.5%)
2	48 (70.6%)	20 (29.4%)	22 (81.5%)	5 (18.5%)	26 (63.4%)	15 (36.6%)
3	22 (53.7%)	19 (46.3%)	10 (47.6%)	11 (52.4%)	12 (60.0%)	8 (40.0%)
4	4 (33.3%)	8 (66.7%)	2 (50.0%)	2 (50.0%)	2 (25.0%)	6 (75.0%)

*Adopted from Table 3 of Batra A. et. al (2021)[29]

Table 11: Distribution of Clinical-Pathologic score and genomic risk category by age group

Clinical-Pathologic model score	Age ≤ 50 years (n = 104)		Age > 50 years (n = 262)	
	Low-risk	High-risk	Low-risk	High-risk
0	60 (98.4%)	1 (1.6%)	109 (100.0%)	0 (0.0%)
1	13 (86.7%)	2 (13.3%)	46 (76.7%)	14 (23.3%)
2	14 (87.5%)	2 (12.5%)	34 (65.4%)	18 (34.6%)
3	7 (63.6%)	4 (36.4%)	15 (50.0%)	15 (50.0%)
4	0 (0.0%)	1 (100.0%)	4 (36.4%)	7 (63.6%)

*Adopted from Table 3 of Batra A. et. al (2021)[29]

Table 12: Sensitivity and specificity analysis of the clinical-pathologic model at various cut-points

Clinical-pathologic model Score	Sensitivity	Specificity	NPV	PPV	Correctly classified
0	56.0%	98.4%	32.1%	99.4%	63.4%
≤ 1	75.5%	73.4%	38.8%	93.1%	75.1%
≤ 2	91.4%	42.2%	50.9%	88.2%	82.8%
≤ 3	98.7%	12.5%	66.7%	84.2%	83.6%

*Adopted from Table 4 of Batra A. et. al (2021)[29]

5.2.4 Safety

No safety evidence available related to molecular profiling for breast cancer.

5.2.5 Economic Implication

There were six economic evaluation studies included in this review. One of it was SR of economic papers and the other five were primary studies of economic evaluation.

Systematic review by Wang SY et. al. in 2018 included 27 studies of economic evaluations of Oncotype DX. Out of 27 studies, 10 studies were from United Kingdom, six from United States and seven from Canada. The SR had a few objectives; first, to identify specific study characteristics and important aspects of genetics/molecular testing economic evaluations and how those characteristics might affect the results, as well as the magnitude of the influence. Second, to examine the frequency of published analyses funded by industry and whether the funding sources associated with study designs which might lead to different conclusions. Third objective was to provide critical insights for value-based framework through appropriately targeting populations for whom Oncotype DX may be most beneficial. The quality of the included studies was assessed using Quality of Health Economic Studies (QHES), generally all the studies had high QHES score (approximately 88 [best score, 100; 95% CI 85 – 90.4]).

The overall findings showed that, in base-case scenario analyses of the included studies, the Oncotype DX had an ICER of ≤$100,000 per QALY. Most of the studies used ≥25-years as timeframe, however, difference in time horizon were not significantly associated with different ICERs. Further assessment on the simulations models, the authors identified eight issues that might compromised the accuracy and validity of the results. The issues were categorised into three main issues; model structure, model assumptions and model input parameters as described in Table 13. Regarding the influenced of funding sources on the study's results; no significant association between the funding sources and the outcome of the studies. Out of 27 studies, 15 studies were directly funded by Genomic Health Industry and the rest of the studies were non-industry funded. As an overall result, either industrial funded or non-funded, the Oncotype DX associated with favourable ICERs; US$900 versus US$3,100 per QALY and more cost saving.[30]

Table 13: Concerning Issues in Existing Oncotype DX Cost-Effectiveness Analysis (CEAs)

Oncotype DX CEAs Issues Categories	Sub-issues	Issues
Model structure	Ignoring clinicopathologic information	Available risk classification models, such as Adjuvant! Online (AOL) and PREDICT risk calculator, could help estimate risks of distant recurrence/breast cancer mortality; ignoring clinicopathologic information would make Oncotype DX more cost effective
	Combining low-, intermediate- and high-risk groups	If one risk group has an ICER > $100,000 per QALY, and other groups have low ICERs, combining low-, intermediate-, and high-risk groups may not reveal the cost-ineffective group
Model assumptions	Oncotype DX decreases chemotherapy use	Limited evidence suggests that Oncotype DX increases chemotherapy use among the clinicopathology-based low-risk group; assuming decrease in chemotherapy use would make Oncotype DX cost effective
	Predictive value of Oncotype DX	Limited evidence supports this assumption, which would favour Oncotype DX cost effectiveness
	Ignoring chemotherapy toxicity	Models that did not include short- or long-term adverse effects attributed to chemotherapy would favour Oncotype DX if Oncotype DX were to increase chemotherapy use but would be against Oncotype DX if Oncotype DX were to decrease chemotherapy use
Input Parameter	Not real-world RS distributions	Existing models generally used data based on 668 patients enrolled in the NSABP B-14 study; HER2 information is not available in this series, and the distributions are not population based; distributions of high-risk RS group would be overestimated, resulting in bias favouring Oncotype DX

Oncotype DX CEAs Issues Categories	Sub-issues	Issues
	Implausible estimates of chemotherapy effectiveness	Some studies selected parameters that chemotherapy increases distant recurrence for the Recurrence Score (RS) low-risk group; these parameters are biologically implausible and would lead to bias favouring Oncotype DX
	Young patient age	Some studies assumed that the age at breast cancer diagnosis is younger than that in the actual population, which could make Oncotype DX cost effective

*Table was adopted from Wang SY et. al.[30]

Previous SR by Blok et. al. included 44 primary economic study consisted of 32 studies on Oncotype DX, seven studies on MammaPrint, one study on EndoPredict and four studies of direct comparison between difference molecular profiling assays. Most of the included studies compared the molecular profiling assays with variety strategies. The evaluations involved five estimated costs (cost-minimization analysis), one estimated life years without QALYs (cost-effectiveness analysis) and 38 estimated QALYs (cost-utility analysis). Out of 44 studies, two studies compared a measured outcome between two actual patient's groups either with or without molecular profiling assays and another 42 studies involved mathematical model to compare the estimated outcomes for different policies. The mathematical models typically estimated a decrease in chemotherapy, a decrease in recurrence and an increase in life years and QALYs. The total healthcare cost may go up or down depended on the balance between the assay costs and saving of chemotherapy and recurrence. Figure 6 tabulated 40 studies of molecular profiling assays versus a strategy without the assays to show the estimated impact of molecular profiling assays on QALYs and costs. The horizontal axis showed the impact on QALYs: all studies but one reported that molecular profiling assays resulted in better patient outcome with a positive impact on QALYs. The vertical axis showed that the impact on costs: the molecular profiling assays was cost saving in 14 (35%) evaluations and cost increasing in 26 (65%) of the evaluations. On average, total costs increase 449 euro per patient with an improvement on patient outcome of 0.16 life years and 0.20 QALYs. In general, there were no apparent differences between estimated outcomes for different molecular profiling assays. The ranges of the costs also comparable in N- and N+ patients but the estimated QALYs gained was larger than in N- (on average, 0.24 versus 0.07 QALYs). Considering the improvement in patient outcome, molecular profiling assays was cost-effective in 36 (90%) of the evaluations (below the dashed 40,000 euro-per-QALYs line).[21]

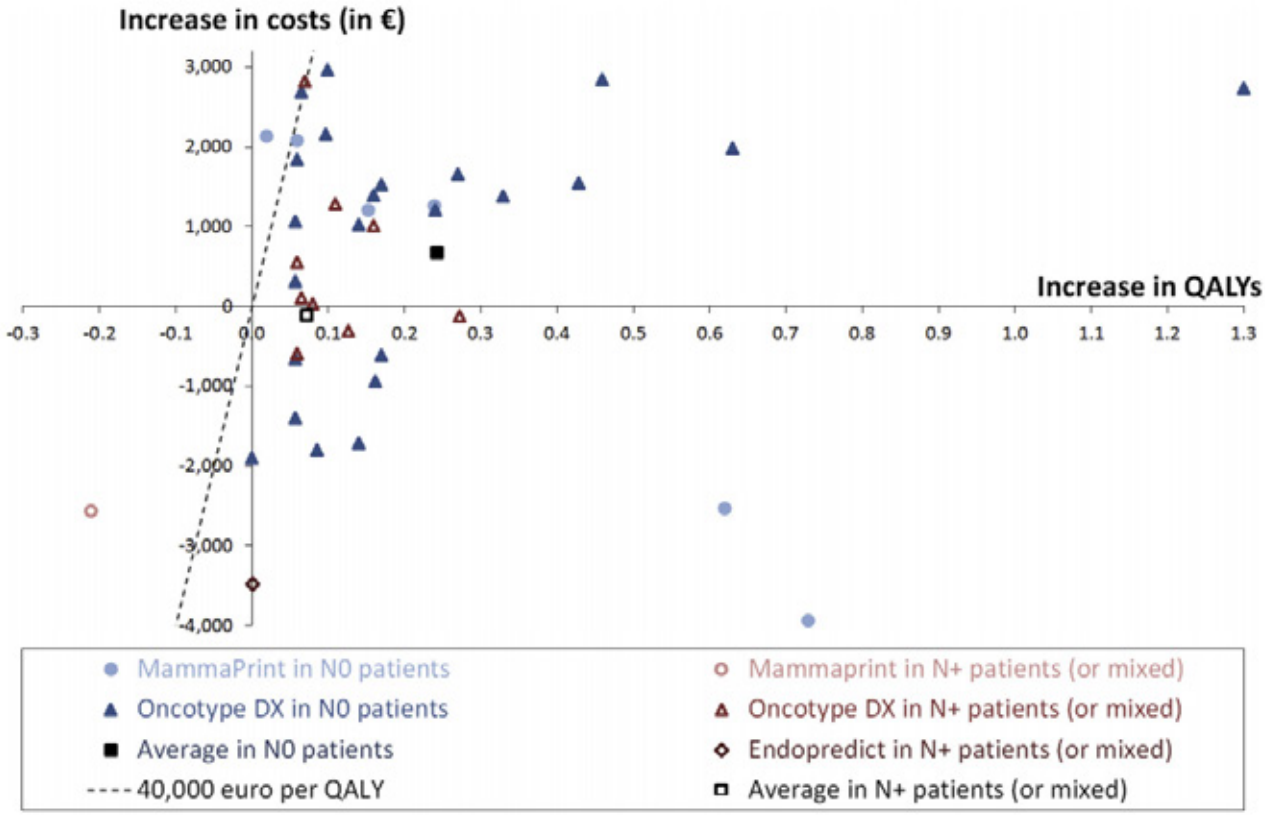
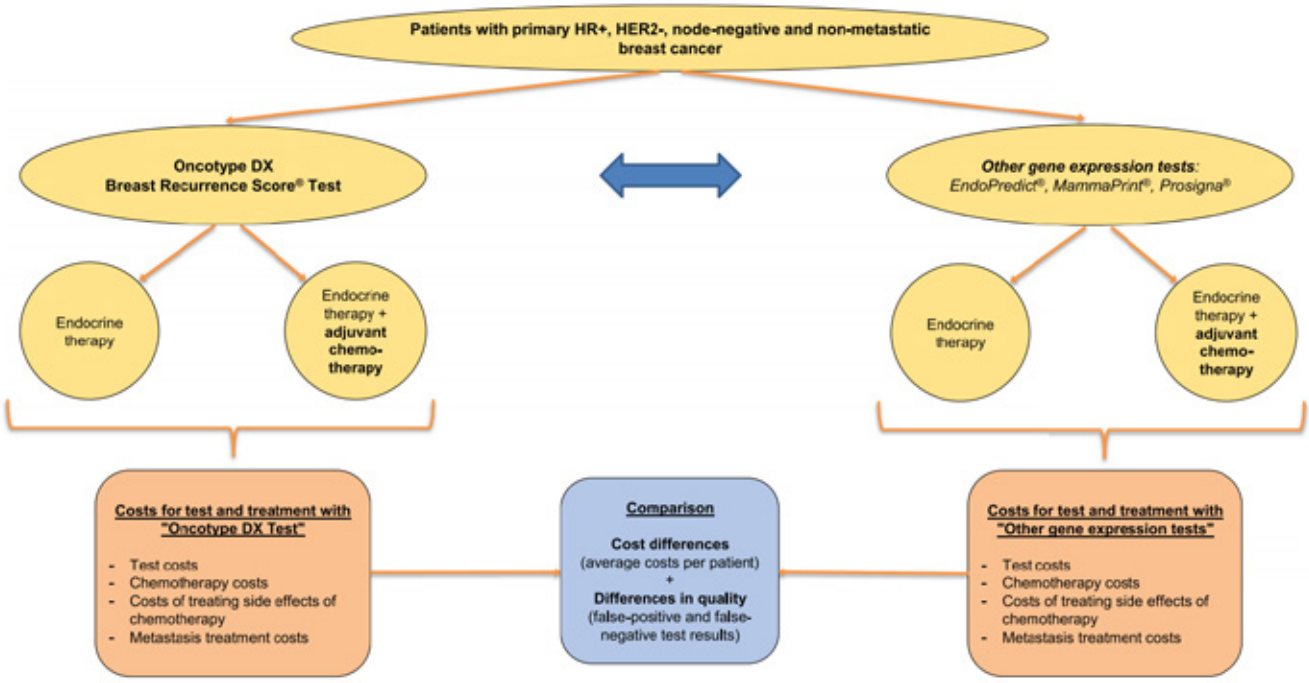


*Figure adopted from Blok et. al.

Figure 6: Estimated impact on cost and quality-adjusted life years (QALYs) per economic evaluation, according to test and nodal status

Lux MP et. al. conducted budget impact analysis (BIA) to compare the available molecular profiling assays and subsequent treatment cost in Germany for HR+, HER2-ve, early breast cancer N- patients and the budgetary impact on the sickness funds. A cost comparison was constructed as an expanded budget impact model to calculate the average total costs per patient covered by public health insurance. For Oncotype DX, TAILORx trial was referred as clinical evidence and was included in IQWiG Report D18-01 which was the basis of granting reimbursement in Germany. In current IQWiG Report D19-01, three other tests were included (EndoPredict, Prosigna and MammaPrint) for further assessment along with the Oncotype DX. Figure 7 showed the overview of the BIA model. The used of Oncotype DX test led to average saving per patient of 2,500€ compared to EndoPredict, 1,936€ compared to MammaPrint and 649€ compared to Prosigna. Although the cost of Oncotype DX test was higher, saving in costs of chemotherapy was due to the fact that EndoPredict and MammaPrint allocated a larger proportion of patients to chemotherapy. The authors also assumed that false-positive (FP) tests lead to an over treatment of chemotherapy with lack of benefit (Incorrect Chemo), so that an unnecessary side effects of the chemotherapy and unnecessary costs for sickness funds would

arise. There was no impact on mortality was assumed in the model by false-positive tests and unnecessary chemotherapy treatment. As for false-negative (FN) tests, patients did not receive a chemotherapy they should have (Incorrect No Chemo), thus increase risk of metastases and mortality, so in this case, chemotherapy initially avoided may save costs, but the treatment of subsequent metastases will lead to increase expenses in the subsequent years. The recurrence risk was estimated to be 14% in patients receiving chemotherapy correctly and 20% in patients falsely not receiving chemotherapy. Thus, from the model saving were achieved by reduction of unnecessary chemo used, a consequence of FP test results (incorrect chemotherapy); 73% in EndoPredict, 42% in MammaPrint, 20% in Prosigna and by assumption of 0% in Oncotype DX. As for FN test results (Incorrect No Chemo); 5% in EndoPredict, 22% in MammaPrint, 49% in Prosigna and 0% by assumption of Oncotype DX reduced necessary chemotherapies, which initially means cost saving but subsequently increase metastases and mortality translating into respective costs. Consequently, the condition led to more late chemotherapies for metastasis carried out for EndoPredict (+100%), and MammaPrint (+49%) and approximately the same for Prosigna (-3%) compared to Oncotype DX test.[31]

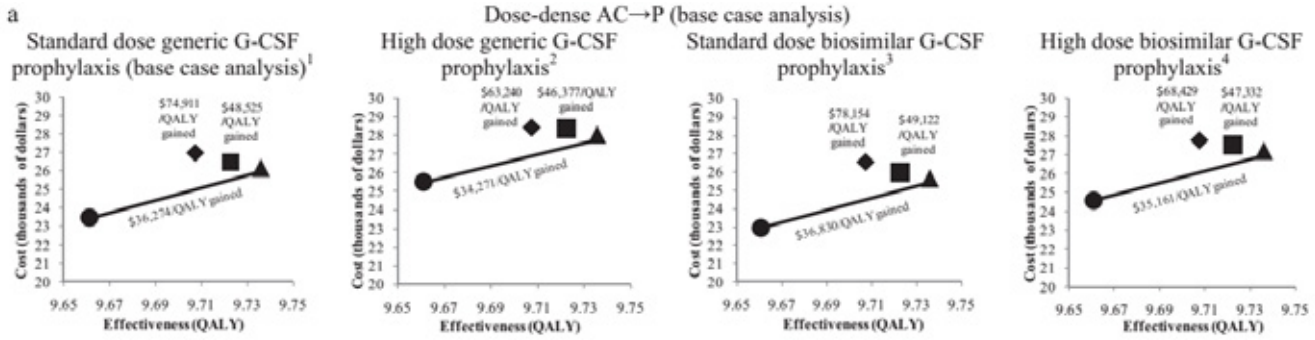


*Adopted from Figure 1 by Lux MP et. al.[31]

Figure 7: Schematic overview of the budget impact model

Hannaouf MB et al. conducted mathematical model to determine the cost-effectiveness of incorporating molecular profiling assays into standard practice using EndoPredict, Prosigna and Oncotype DX compared head-to-head and to traditional clinical-pathological predictors alone to guide adjuvant therapy decisions in women with LN-, HR+, HER2-ve early-stage breast cancer from the perspective of the Canadian public healthcare system. The authors developed two models; model A treated with endocrine alone and model B treated with both endocrine and chemotherapy. For model B, different chemotherapy with serious adverse events were associated with different mortality risk, different quality of life and different management cost. The discount rate applied was 1.5% per annum to the costs and QALYs as recommended by CADTH. Compared to clinical-pathological predictors alone-based strategy, addition of Oncotype DX yielded $74, 911 per QALY gained, yielded $36, 274 per QALY gained with EndoPredict and $48, 525 per QALY gained with Prosigna. Figure 8 illustrates differences in costs and effects between the four model strategies using a cost-effectiveness plane showed that, Prosigna was cost saving compared to Oncotype DX and the EndoPredict was the dominating strategy. From deterministic sensitivity analysis, after considering acceptable adjuvant chemotherapy, Prosigna remained cost-saving compared to Oncotype DX and EndoPredict remained the dominating strategy. Discounting was available for the molecular profiling up to 50% resulted in decreased differences between ICERs within a range of $15,000 to $ 11,000 per QALY gained. The sensitivity analyses showed that chemotherapy administration and utility, risk of death and cost associated with chemotherapy related adverse events did not substantially influence the baseline results. From probabilistic sensitivity analysis comparing four model strategies showed that with willingness to pay threshold of $100,000 per QALY gained, the analysis found that the preferred strategies percentage were 15% for clinical-pathological alone, 45% for EndoPredict plus clinical-pathological predictors, 29% for Prosigna plus clinical-pathological predictors and 11% for Oncotype DX plus clinical-pathological predictors. The authors also performed ‘value-of-information analyses’, to estimate the expected value of removing all statistical uncertainty of the three molecular profiling tests related parameters including risk classification, chemotherapy administration and 10-year risk of distant recurrence. Using the baseline ICER value of $74,911 per QALY gained of Oncotype DX as the willingness to pay, the opportunity cost associated with the choice of an optimal molecular profiling-based strategy to guide adjuvant therapy resulted in a total expected value of partial perfect information (EVPPI) of $2,195 per woman with LN-, HR+, HER-ve early-stage breast cancer. The total EVPPI for the entire LN- HR+ HER2-ve early-stage breast cancer

population that could be eligible for gene expression profiling testing in Canada was 4,738 eligible patients with LN-, HR+ HER2-ve early-stage breast cancer per year that resulted to a total of $10.4 million per year ($2,195 per patient x 4,738 = $10.4 million per year).[32]



■ Prosigna + CP predictors; ▲ EndoPredict + CP predictors; ♦ Oncotype DX + CP predictors; ● CP predictors alone
*Figure adopted from Figure 2a Hannouf MB et. al.[32]

Figure 8: Outcomes of the decision model by type of adjuvant chemotherapy regimen

Ramirez SP et. al. evaluated the prospective, multicentre program called PREGECAM involving women with early-breast cancer from 21 hospitals in Madrid. The primary aims of PREGECAM were to prospectively evaluate the impact of Oncotype DX and MammaPrint on adjuvant decision making and to assess the cost-effectiveness of both molecular profiling assays with traditional prognostic factors. The secondary objectives were to examine the association between clinical-pathological markers and likelihood of change in treatment recommendations. A total of 907 patients involved in the analysis consisted of patient with operable breast cancer, ER+/HER2-ve by IHC or FISH, tumour size ≥ 1. Before molecular profiling assays were conducted, the involved oncologists were required to complete pre-test questionnaires that recorded initial treatment recommendation solely based on standard prognostic factors. After that the oncologists will place order for either Oncotype DX or MammaPrint according to their preferences. Subsequently the results of molecular profiling tests available, once again the oncologist will complete other questionnaires to state their final treatment recommendation; the recommendation need to be detailed in terms of chemotherapy and endocrine therapy inclusive any specific agents to be administered to the patients. As for pharmacoeconomic models, the economic model developed with decision tree and Markov model and the data on probabilities of treatment recommendations were taken from PREGECAM. Table 14 and Table 15 showed the treatment recommendations from pre- to post-molecular profiling assays tests. Based on the table, initial treatment recommendations were revised in 42.6% of all assessable patients. The changes from pre-test recommendation

of chemotherapy to post-test recommendation of endocrine therapy in 277 patients (30.5%). Meanwhile, changes from pre-test recommendation of endocrine therapy to post-recommendation of chemotherapy in 109 patients (12%). According to individual test, post-Oncotype DX test showed 196 out of 440 patients changed their treatment recommendations; 152 patients (34.5%) changed from chemotherapy to endocrine therapy alone and another 44 patients (10.0%) changed from endocrine therapy alone to chemotherapy. In addition, the changes of post-Oncotype DX treatment recommendation were consistent with the Recurrence Score. As for MammaPrint, changes in treatment recommendation were seen in 190 patients out of 467 (40.7%). The post-assay recommendation remained consistent with the risk results; 125 patients (26.85%) had revised the initial recommendation from chemotherapy to endocrine therapy and another 65 patients (13.9%) was changed from endocrine therapy to chemotherapy. The recurrence risk provided by both Oncotype DX and MammaPrint was significantly associated with likelihood of change in treatment recommendations ($p < 0.001$). For association between clinicopathological variables and the likelihood of change in treatment recommendation after molecular profiling assays tests, logistic regression was performed. The analysis found that there were significant association of lower tumour grade ($p < 0.001$), higher levels of PR positivity ($p < 0.001$) and low Ki-67 expression ($p < 0.001$) towards changing from chemotherapy to endocrine therapy. Meanwhile for changing from endocrine therapy to chemotherapy, the association was significant towards higher tumour grade ($p = 0.03$), lower levels of PR positivity ($p = 0.005$) and high Ki-67 expression ($p < 0.001$).[33]

Table 14: Treatment recommendation before and after Oncotype DX test results

Pre- to post-Oncotype DX® treatment recommendation	Low RS (<18) *N*=238 *n* (%)	Intermediate RS (18–30) *N*=168 *n* (%)	High RS (>30) *N*=34 *n* (%)	Total *N*=440 *n* (%)
Treatment plan changed	127 (65)	63 (32)	6 (3)	196 (45)
HT–CHT	0 (0)	38 (86)	6 (14)	44 (10)
CHT–HT	127 (84)	25 (16)	0 (0)	152 (35)
Treatment plan not changed	111 (45)	105 (43)	28 (11)	244 (55)
CHT–CHT	7 (6)	85 (71)	28 (23)	120 (27)
HT–HT	104 (84)	20 (16)	0 (0)	124 (28)

RS recurrence score, *HT* hormone therapy, *CHT* chemohormonal treatment

*Table adopted from Table 2 of Ramirez et. al.[33]

Table 15: Treatment recommendation before and after MammaPrint test result

Pre- to post-MammaPrint® treatment recommendation	Low risk *N*=297 *n* (%)	High risk *N*=170 *n* (%)	Total *N*=467 *n* (%)
Treatment plan changed	125 (66)	65 (34)	190 (41)
HT–CHT	0 (0)	65 (100)	65 (14)
CHT–HT	125 (100)	0 (0)	125 (28)
Treatment plan not changed	172 (62)	105 (38)	277 (59)
CHT–CHT	4 (4)	105 (96)	109 (23)
HT–HT	168 (100)	0 (0)	168 (36)

HT hormone therapy, *CHT* chemohormonal treatment

*Table adopted from Table 3 Ramirez et. al.[33]

In deterministic analyses, the authors found that cost per patient from national health system and societal perspective compared to clinical practice were lower with molecular profiling assays which were 13,867€ and 32,678€ respectively. The reallocation of adjuvant chemotherapy based on test result was associated with improvement of 0.00787 QALYs per patient where both tests were found to dominate over standard care. Based on probabilistic analysis, probability of saving costs with molecular profiling assays was 100%; from national health system perspective by 13,920€ (95% CI 11,697€ - 12,218€), and from societal perspective by 32,793€ (95% CI 28,432€ - 37,827€). Thus, the probability of cost-effectiveness (for willingness to pay 30,0000€ per QALY gained) was 78.5% for national health system perspective and 78% for societal perspective.[33]

Ozmen V et. al. conducted another cost-effectiveness study to determine the costs of chemotherapy in government hospitals in Turkey and evaluated the cost-effectiveness of the Oncotype DX from national insurance perspective. The study also evaluated the cost-effectiveness of Oncotype DX in developing country using Turkish population as a model difference. Base case analysis showed that the Oncotype DX was projected to cost an additional $1.492 per patients compared with current clinical practice over a 30-year time horizon ($5.141 versus $3.649). The costs increment was associated with an improvement in life expectancy of 0.86 years (24.84 years versus 25.70 years) and an increased in quality-adjusted life expectancy of 0.68 QALYs (19.26 QALYs versus 19.94 QALYs). The ICERs was estimated to be $7,207.9 per QALY gained and 5,720.6 per LY gained for Oncotype DX versus current clinical practice in Turkey. According to one-way sensitivity analysis, the base-case

outcomes were most sensitive to variation in age, cost of Oncotype DX and change in chemotherapy recommendation for low-risk patients. An increase in the baseline age of patients in the simulation by 25%; increased the ICER for Oncotype DX versus current care to $7,971.72 per life years (LY) gained due to competing mortality because patients were not alive long enough to accumulate the full benefit of the Oncotype DX. In contrast, reducing the baseline age improved the cost-effectiveness of Oncotype DX ($5,213.70 per LY gained).[34]

Hall PS. et. al. conducted further analysis on OPTIMA trial to evaluate the performance of health economics of alternative molecular profiling assays to determine which assays to be evaluated in subsequent main trial, and to establish acceptability among patients and clinicians. The OPTIMA trial recruited 313 patients (68% were postmenopausal women) and 302 of them had samples that available for molecular profiling assays test. From the assays test, proportion of patients considered as low-risk by each test and potentially spared chemotherapy ranged from 0.82 (Oncotype DX) to 0.55 (IHC4-AQUA) (Table 16). On the intended chemotherapy regimen for each patient and proportion allocated to high- or low-risk by each test, the expected mean costs of chemotherapy ranged from £3611 per patient (all patients treated with chemo) to £2102 per patient (Prosigna ROR). The correlation between 10-year predicted recurrence-free survival and test scores were 0.24 in Oncotype DX, 0.36 in Prosigna ROR, 0.17 IHC4 and 0.14 for IHC4-AQUA. In base-case analysis, the expected lifetime per patient cost if all patient received chemotherapy was £13, 961 (95% CI £10,535 - £21,203) with expected lifetime QALYs of 7.69 (95% CI 5.06 – 9.58). Meanwhile for individual testing, mean incremental QALYs with each testing strategy were very similar; between 0.17 and 0.20 more than chemotherapy for all, although credible intervals were generally around ± QALY (Table 17). The mean incremental cost per patient also varied, between an additional cost of 195 (95% CI -£3260 to £3430) with MammaPrint to a saving of £1892 (95% CI -£5415 to £1488) with IHC4 in comparison with all patients received chemotherapy. The authors reported that the net health benefit from all assays strategies was higher than standard care, although it was very similar magnitude between assays. Further analysis showed that uncertainty in the cost-effectiveness of all test was large. The probability of individual tests was more cost-effective than standard care ranged from 75% (MammaPrint) to 81% (IHC4) in separate 2-way comparisons. The incremental analysis found that the probability that test-directed chemotherapy using any test was more cost-effective than standard care was 86%. In order to plan for future planned research, the authors generated' value of information analyses based on theory that if evidence

for the effectiveness or cost-effectiveness of a new technology is uncertain, the researcher is in risk making a suboptimal decision about which to adopt for populations used. The consequences of such decision are lost health or lost resources compared with the optimal decision. Reduction in decision uncertainty therefore had quantifiable value and the results are presented in expected net health benefit, expected value of perfect information (EVPI), expected value of perfect parameter information (EVPPI) and expected value of sample information (EVSI). Sensitivity analysis showed that treating all patients with chemotherapy was more cost-effective than any of the testing option with probability of individual tests being cost-effective ranging between 31% and 50%. Population EVPI was 4165 QALYs, QALYs, suggested that further research may be worthwhile even if the chemo effect is thought to be constant. The sensitivity analysis found that Oncotype DX was a favoured on the basis of expected cost-effectiveness followed with Prosigna ROR.[35]

Table 16: Costs of each testing strategy, proportion allocation to high-risk group, and expected chemotherapy costs in OPRIMA prelim

Testing strategy	Proportion low-risk (spared chemotherapy)*	Testing cost per-patient (£) (95% CI)	Mean chemotherapy cost per-patient† (£)	Forecast mean 10-y recurrence-free survival (%)‡	
				Low-risk	High-risk
Chemotherapy for all	0		3611	59.8	
Oncotype DX	0.82	2580 (fixed)	678	60.9	54.6
MammaPrint	0.61	2207 (fixed)	1409	61.6	57.0
Prosigna		1672.50 (1576–1773)			
Subtype	0.59		1509	62.4	55.9
ROR	0.65		1291	61.8	55.9
MammaTyper	0.62	1277§ (186–6415)	1422	61.1	57.5
IHC4-AQUA	0.55	720 (fixed)	1610	60.6	58.7
IHC4	0.61	152 (61–322)	1370	60.5	58.5

CI, confidence interval; OPTIMA, Optimal personalised treatment of breast cancer using multi-parameter analysis.
* Patients with unavailable test results are assumed to be high-risk and are treated with chemotherapy.
† Average per-patient procurement and delivery costs based on prescribing intent and test assignment and not including costs of toxicity.
‡ Forecast using Adjuvant! Online (treated with hormone therapy but no chemotherapy).
§ Unavailable from manufacturer and therefore estimated by analyst.

*Adopted from Table 2 of Hall PS et. Al (2017)[35]

Table 17: Cost-effectiveness results – Incremental analysis in comparison with all patients receiving chemotherapy

	Oncotype DX	MammaPrint	Prosigna Subtype	Prosigna ROR	MammaTyper	IHC4-AQUA	IHC4
Base-case analysis							
Mean incremental QALYs per person (95% CI)	0.2 (−1.07 to 1.4)	0.18 (−0.87 to 1.1)	0.18 (−0.85 to 1.05)	0.18 (−0.91 to 1.15)	0.18 (−0.95 to 1.15)	0.17 (−0.87 to 1.05)	0.18 (−0.93 to 1.14)
Mean incremental cost per person (£) (95% CI)	−108 (−4610 to 4292)	195 (−3206 to 3430)	−281 (−3553 to 2774)	−474 (−4078 to 2955)	−944 (−4481 to 2380)	−1115 (−4373 to 1943)	−1892 (−5415 to 1488)
ICER (£ per QALY)	Dominates*	1097	Dominates*	Dominates*	Dominates*	Dominates*	Dominates*
Probability that test is cost-saving	0.53	0.39	0.62	0.68	0.80	0.84	0.90
Probability that test provides more benefit	0.73	0.73	0.74	0.73	0.73	0.73	0.73
Probability that test is cost-effective	0.77	0.75	0.77	0.77	0.78	0.79	0.81
Incremental net benefit (QALYs) (95% CI)†	0.21 (−0.87 to 1.21)	0.17 (−0.74 to 0.94)	0.19 (−0.71 to 0.93)	0.21 (−0.76 to 1.01)	0.23 (−0.77 to 1.04)	0.23 (−0.69 to 0.97)	0.27 (−0.69 to 1.08)
Sensitivity analysis: Constant relative chemotherapy effect							
Probability that test is cost-effective	0.33	0.31	0.41	0.35	0.36	0.50	0.43
Incremental net benefit vs. chemotherapy for all (QALYs) (95% CI)*	−0.09	−0.08	−0.05	−0.05	−0.03	−0.01	−0.02
Sensitivity analysis: Variable survival after recurrence							
Probability that test is cost-effective vs. chemotherapy for all	0.97	0.94	0.94	0.94	0.94	0.94	0.95
Incremental net benefit vs. chemotherapy for all (QALYs) (95% CI)*	0.70	0.54	0.54	0.60	0.61	0.58	0.66

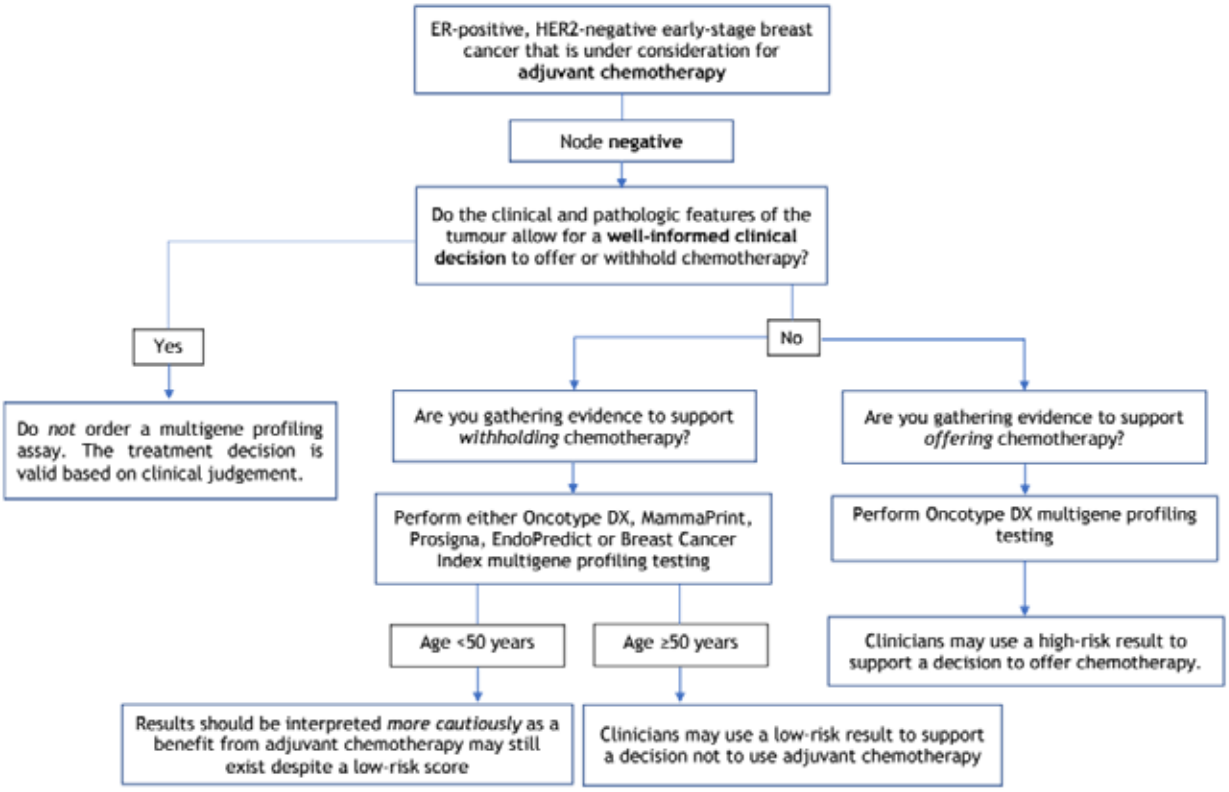
CI, confidence interval; ICER, incremental cost-effectiveness ratio; QALY, quality-adjusted life-year.
* "Dominates" implies that the test is more effective and less costly than all patients receiving chemotherapy.
† A positive incremental net benefit is necessary for a test to be considered more cost-effective than all patients receiving chemotherapy. The higher the incremental net benefit, the more cost-effective the test is expected to be.

*Adopted from Table 3 of Hall PS et. Al (2017)[35]

5.2.6 Organisational & Guidelines

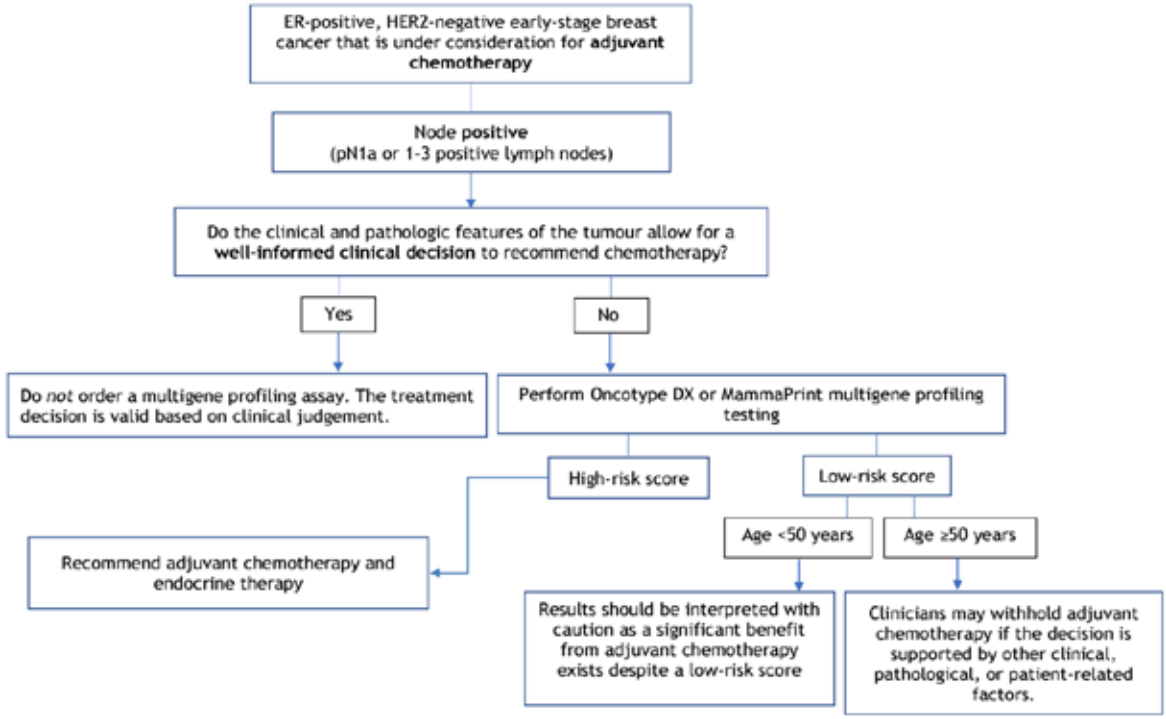
Ontario Health (Cancer Care Ontario)'s Program in Evidence-Based Care (PEBC) developed a guideline on molecular profiling assay of breast cancer which were recently published in 2022. The purpose of the guideline was to determine the clinical utility of molecular profiling assays (Oncotype DX, MammaPrint, Prosigna, EndoPredict and Breast Cancer Index) in individuals with early-stage invasive breast cancer. The authors of this guideline were among experts in medical oncology, pathology, medical genetics and health research methodology. They conducted an SR to assess the clinical utility of the molecular profiling assays in terms of the ability to predict response to adjuvant chemotherapy and extended adjuvant endocrine therapy. The review also aimed for evidence on used of the assays in the setting of either node-negative or node-positive ER+/HER2-ve breast cancer patients to guide clinical decisions to withhold or offer adjuvant chemotherapy. On the other hand, patient factors impacting the utilisation of molecular profiling results also being reviewed. The PEBC produced the evidence-

based and evidence-informed guidance documents using the methods of the Practice Guidelines Development Cycle which involved a systematic review with the interpretation of the evidence by the authors who then drafted recommendations based on the evidence and expert consensus. This review also involved patients/survivors/caregivers as Consultation Group Member. The target population for this guideline was individual with early-stage invasive breast cancer who actually required further information for prognosis and treatment decision making. The early-stage invasive breast cancer is defined as stage I to III breast cancers that were surgically operable and do not have evidence of locally recurrent or distant metastatic disease with pT1 -T3 or pN0-N1a based on surgical pathologic staging. The intended users of this guideline were clinicians and policymakers involved in the diagnosis and treatment of breast cancer. For Recommendations 1, clinicians should consider used of any five assays to help guide the use of systemic therapy in patients with early-stage ER+/HER2-ve breast cancer. Next, Recommendation 2, clinician may use a low-risk results from all five assays to support a decision not to use adjuvant chemotherapy in patients with early-stage node-negative ER+/HER-ve disease. Figure 9a, 9b and 9c were summary of the recommendations in a decision tree. In Recommendation 3, clinicians may use a high-risk result from Oncotype DX to support a decision to offer chemotherapy in patients with node-negative ER+/HER2-ve. For Recommendation 4, clinicians may withhold chemotherapy based on a low-risk Oncotype DX or MammaPrint score if the decision is supported by other clinical, pathological or patient-related factors in post-menopausal patient with ER+/HER2-ve tumours and one to three nodes involved (N1a disease). The last recommendation, Recommendation 5 stated that, clinicians may consider the used of Breast Cancer Index (H/I) high assay result to support a decision to extend adjuvant endocrine therapy if the decision is supported by other clinical, pathological or patient-related factors in patients with ER+ disease.[15]



*Adopted from figure 1 of Blanchette P. et. al.[15]

Figure 9a: Molecular Profiling Assays Decision Tree for Adjuvant Chemotherapy in Node-Negative patients



*Adopted from figure 2 of Blanchette P. et. al.[15]

Figure 9b: Molecular Profiling Assays Decision Tree for Adjuvant Chemotherapy in Node-Positive patients

Table 16: Costs of each testing strategy, proportion allocation to high-risk group, and expected chemotherapy costs in OPRIMA prelim

Testing strategy	Proportion low-risk (spared chemotherapy)*	Testing cost per-patient (£) (95% CI)	Mean chemotherapy cost per-patient† (£)	Forecast mean 10-y recurrence-free survival (%)‡	
				Low-risk	High-risk
Chemotherapy for all	0		3611	59.8	
Oncotype DX	0.82	2580 (fixed)	678	60.9	54.6
MammaPrint	0.61	2207 (fixed)	1409	61.6	57.0
Prosigna		1672.50 (1576–1773)			
Subtype	0.59		1509	62.4	55.9
ROR	0.65		1291	61.8	55.9
MammaTyper	0.62	1277§ (186–6415)	1422	61.1	57.5
IHC4-AQUA	0.55	720 (fixed)	1610	60.6	58.7
IHC4	0.61	152 (61–322)	1370	60.5	58.5

CI, confidence interval; OPTIMA, Optimal personalised treatment of breast cancer using multi-parameter analysis.
* Patients with unavailable test results are assumed to be high-risk and are treated with chemotherapy.
† Average per-patient procurement and delivery costs based on prescribing intent and test assignment and not including costs of toxicity.
‡ Forecast using Adjuvant! Online (treated with hormone therapy but no chemotherapy).
§ Unavailable from manufacturer and therefore estimated by analyst.

*Adopted from Table 2 of Hall PS et. Al (2017)[35]

Table 17: Cost-effectiveness results – Incremental analysis in comparison with all patients receiving chemotherapy

	Oncotype DX	MammaPrint	Prosigna Subtype	Prosigna ROR	MammaTyper	IHC4-AQUA	IHC4
Base-case analysis							
Mean incremental QALYs per person (95% CI)	0.2 (−1.07 to 1.4)	0.18 (−0.87 to 1.1)	0.18 (−0.85 to 1.05)	0.18 (−0.91 to 1.15)	0.18 (−0.95 to 1.15)	0.17 (−0.87 to 1.05)	0.18 (−0.93 to 1.14)
Mean incremental cost per person (£) (95% CI)	−108 (−4610 to 4292)	195 (−3206 to 3430)	−281 (−3553 to 2774)	−474 (−4078 to 2955)	−944 (−4481 to 2380)	−1115 (−4373 to 1943)	−1892 (−5415 to 1488)
ICER (£ per QALY)	Dominates*	1097	Dominates*	Dominates*	Dominates*	Dominates*	Dominates*
Probability that test is cost-saving	0.53	0.39	0.62	0.68	0.80	0.84	0.90
Probability that test provides more benefit	0.73	0.73	0.74	0.73	0.73	0.73	0.73
Probability that test is cost-effective	0.77	0.75	0.77	0.77	0.78	0.79	0.81
Incremental net benefit (QALYs) (95% CI)†	0.21 (−0.87 to 1.21)	0.17 (−0.74 to 0.94)	0.19 (−0.71 to 0.93)	0.21 (−0.76 to 1.01)	0.23 (−0.77 to 1.04)	0.23 (−0.69 to 0.97)	0.27 (−0.69 to 1.08)
Sensitivity analysis: Constant relative chemotherapy effect							
Probability that test is cost-effective	0.33	0.31	0.41	0.35	0.36	0.50	0.43
Incremental net benefit vs. chemotherapy for all (QALYs) (95% CI)*	−0.09	−0.08	−0.05	−0.05	−0.03	−0.01	−0.02
Sensitivity analysis: Variable survival after recurrence							
Probability that test is cost-effective vs. chemotherapy for all	0.97	0.94	0.94	0.94	0.94	0.94	0.95
Incremental net benefit vs. chemotherapy for all (QALYs) (95% CI)*	0.70	0.54	0.54	0.60	0.61	0.58	0.66

CI, confidence interval; ICER, incremental cost-effectiveness ratio; QALY, quality-adjusted life-year.
* "Dominates" implies that the test is more effective and less costly than all patients receiving chemotherapy.
† A positive incremental net benefit is necessary for a test to be considered more cost-effective than all patients receiving chemotherapy. The higher the incremental net benefit, the more cost-effective the test is expected to be.

*Adopted from Table 3 of Hall PS et. Al (2017)[35]

5.2.7 Social, ethical and legal issues

Ramirez SP et. al. collected a survey among PREGECAM patients who underwent the molecular profiling assays test. They were required to complete a questionnaire in order to assess their knowledge and personal opinion regarding to the role of molecular profiling test in early-stage breast cancer treatment. Fifty-nine patients from single institution participated in the study; 27 patients (46%) were highly educated and 83% of them did not undergo adjuvant chemotherapy. From the assessment, only nine patients (15%) were aware about the existence of molecular profiling assays before they were offered the assays by their oncologists. Almost all the patients (57 patients; 97%) feel more confident with their final treatment recommendation after the assays test.[33]

5.3 DISCUSSION

Molecular profiling assays among early-stage breast cancer patients play an important role in overall management. Based on the three included SR, molecular profiling assays results provided comparable prognostic information in women ≤40 years old with elderly counterparts. Based on the SR, both Oncotype Dx and MammaPrint were good prognostic test for LN+ and LN- patients either in low- or high-risk score group and EndoPredict was specifically for menopausal women. The Oncotype DX was able to classify high-risk LN- patients into low-risk without chemotherapy with excellent prognosis. Either in LN- or LN+ Oncotype Dx showed good predictive results. An HTA by Ontario in 2020 also reported that, Oncotype DX was a good prognostic and predictive test for both LN- and LN+. However, the result was lower for LN+.[39] The SR reported that Oncotype DX was also a reliable prognostic test in high-risk score patients treated with chemotherapy regardless of N0 or N1 breast cancer. The Oncotype Dx score also significantly correlated with DFS and OS and was good predictive in chemotherapy especially in high-risk score groups. Even changes in treatment decision either escalated or deescalated after the assays test also significant in both Oncotype DX and MammaPrint but studies that observed the benefit of chemotherapy after the changes were not conducted. According to HTA by Harnan S. et. al., the evidence as predictive of chemotherapy benefit were limited and varied.[13]

On the other hand, clinicopathological factors (CP) also play an important role in treatment decisions along with the molecular profiling assay results. The included studies showed that patients with low-risk scores will require chemotherapy after further assessment with CP

factors. In one SR, the Oncotype DX score reduced the chemotherapy decision in N+ population from 70% to 20%.

Actually, there was no prospective trial comparing between assays retrieved. However, limited number of studies looked the concordance and correlation between the assays as well as correlation with clinicopathological factors. Correlation among Oncotype DX and MammaPrint was reported in two big cohorts (> 100,000 patients) which showed that both assays had similar prognostic ability in identification of low-risk individual who could spared chemotherapy. Besides, both assays significantly reduced rate of chemotherapy administration. On the other hand, MammaPrint was mostly used among stage II and stage III patients especially those with ER-, HER- and LN+ as the risk score result was consistent with post-operative chemotherapy decision. Meanwhile, the Oncotype DX was widely used among stage I and less-operative chemo administration was reported. Another correlation was between Oncotype DX and Prosigna, two included cohorts showed that both assays had very weak correlation among each other even in the postmenopausal population. One study comparing both Oncotype DX and Prosigna results with clinicopathological model. The study was developing a clinicopathological model in order to narrow down the breast cancer population who might gained more benefit from the assays test. based on the model, larger tumour size, high-grade tumour and lower expression of PR were higher in high-risk score group than low-risk score group. One study reported that MammaPrint and EndoPredict had overall significant fair agreement, but not in high-risk cases as EndoPredict scored significantly more high-risk score than MammaPrint. There was significant association between molecular profiling assays risk score with tumour grade and Ki-67. Based on the variation in outcome of the molecular profiling assays, they should not be used interchangeably.

One SR also reported on economic studies of the molecular profiling assays. The studies showed that molecular profiling assays were cost-effective and the economic model showed that the assays resulted in better patient outcome with positive impact on QALYs. According to the SR, it was a comparable cost between N- and N+ but the estimated QALYs gained was larger in N-. The Ontario HTA also reported that molecular profiling assays were generally cost-effective compared to usual care among ER+, LN- and HER2- breast cancer patients and was likely cost-effective in LN+ and premenopausal women. Another HTA by Harnan S. et. al. also reported that some test may have a favourable cost-effectiveness profile for certain patient

subgroups; the HTA suggested that incremental cost-effectiveness ratios for the test versus current practice were broadly favourable for the following scenarios: (1) Oncotype DX, for the LN0 subgroup with a Nottingham Prognostic Index (NPI) of > 3.4 and the one to three positive lymph nodes (LN1–3) subgroup (if a predictive benefit is assumed); (2) IHC4 plus clinical factors (IHC4+C), for all patient subgroups; (3) Prosigna, for the LN0 subgroup with a NPI of > 3.4 and the LN1–3 subgroup; (4) EndoPredict Clinical, for the LN1–3 subgroup only; and (5) MammaPrint, for no subgroups.

One SR of economic evaluations of on the Oncotype DX reported possibility of generated ICERs in economic models might be overestimated or underestimated due to inaccurate/not evidence-based assumptions. No matter what, the SR also found that the Oncotype DX was cost effective with the ICER ≤100,000 per QALY. Even budget impact analysis in Germany showed that the Oncotype DX reduced the cost of healthcare with no negative impact on mortality when compared with EndoPredict and MammaPrint. Meanwhile, at the Canadian public healthcare system perspective, addition of molecular profiling assays into clinicopathological predictors to guide chemotherapy decision was cost-effective, and they found that Prosigna and EndoPredict was more cost-effective than the Oncotype DX. However, they highlighted that for routine used, they warranted more comparative field evaluations. In UK study, Prosigna was the preferred assays for further research, however, in sensitivity analysis the Oncotype DX was a favoured assays on the basis of expected cost-effectiveness followed with the Prosigna. In Spain, Oncotype DX and MammaPrint play a significant role in treatment management of patients with early-stage breast cancer and both assays were cost-saving and highly cost-effective at national health care system and societal perspective. In Turkey, the Oncotype DX was found to be cost-effective at national health care perspective with improvement in QoL and may be introduced for routine clinical practice among early breast cancer patients.

Limitations

The authors acknowledge some limitations in the review and these should be considered when interpreting the results. Although there was no restriction in language during the search, only the full text articles in English published in peer-reviewed journals were included in the report, which may have excluded some relevant articles and further limited our study numbers. One of the important limitations was the methodological quality of the included studies, particularly

in terms of heterogeneity, sample size and the risk of bias. This could due to the differences in the baseline characteristics of the study participants, differences in the inclusion and exclusion criteria of each study, assessment of outcomes, and the differences among the molecular profiling assays itself.

6.0 PART B: ECONOMIC EVALUATION

The general objective of this economic evaluation was to assess the cost benefit of using new molecular profiling assays in guiding decision making on chemotherapy treatment for early HR-positive HER2-negative breast cancer patients.

The specific objectives were to estimate the savings associated with the usage of new molecular profiling assays compared to conventional clinical risk prognostic tools in decision making on chemotherapy for HR-positive HER2-negative node negative (N0) as well as node positive (N1-3) in early breast cancer patients; and to estimate the budget implicated for the population that would benefit from the cost savings.

6.1 METHODS

A decision tree model was developed with Microsoft 365 Excel Workbook® to estimate the costs and benefit of using molecular profiling assays for chemotherapy guidance in early HR-positive HER2-negative breast cancer compared with using conventional non-genetic risk prognostic tools (St Gallens classification, PREDICT online, Adjuvant! Online) alone. The perspective taken was from the Ministry of Health perspective.

St Gallens Classification is a tool used to risk stratify breast cancer patients regardless of type, taking into account tumor size, HR status, age, grade, peritumoral vascular invasion, HER2 expression, and Ki-67, described in the CPG Management of Breast Cancer (3rd Edition).

PREDICT and Adjuvant! online are tools which are able to generate a survival estimate based on the clinicopathological data keyed in.

Those included in the simulation cohort were the HR- positive, HER2- negative early breast cancer with either LN- negative (No node involvement) or LN-positive (one to three node involvement) who have undergone surgery.

Based on the systematic review and meta-analysis conducted in this HTA report earlier, molecular profiling assays (regardless the type of assays) was cost effective in 90% of economic evaluation studies, with estimated QALYs gained larger in the node-negative group. Regardless of lymph node status, Oncotype DX and MammaPrint was able to predict the potential benefit to be seen with omission or administration of chemotherapy. For the purpose of this cost benefit analysis, the Oncotype DX and MammaPrint tests were simulated in the model as the locally available interventional gene expression profile assays, and the comparator was the conventional non-genetic risk prognostic tools.

The short-term outcome was measured as cost benefit from chemotherapy averted.

6.1.1 Model Structure

The model structure was constructed following a literature review, and consultation with an expert committee which consisted of multidisciplinary experts namely clinical oncologists, breast and endocrine surgeons, pathologists, radiologists, health economists, public health physicians and pharmacists. This economic evaluation was designed from the Ministry of Health (MOH) perspective.

The patient cohort is simulated into the molecular profiling assay arm and conventional test arm risk stratification into low, intermediate, and high risk of recurrence (for Oncotype DX), or low and high risk of recurrence (for MammaPrint).

A hypothetical cohort of early HR-positive HER2-negative breast cancer patients were simulated in two strategies compared to conventional non-genetic risk prognostic tool with two subgroups each. The same model was simulated for both subgroups of each subgroup.

i) Molecular profiling assays – Oncotype DX (Subgroups LN- and LN+)

ii) Molecular profiling assays - MammaPrint (Subgroups LN- and LN+)

6.1.2 Model Estimation

The epidemiological and disease-related data were obtained from local sources of data whenever available, or literature review when local data was not available. The proportion of patients in each risk level is taken from literature review, while the cost of treatment was from local institution data. The hypothetical cohort was derived from mixed local registry data and literature review.

The estimation of cohort was based on the new breast cancer cases per year data in 2020, proportion of early breast cancer patient, proportion of HR-positive HER2-negative breast cancer patients.

Table 18. Cohort size estimation data

Variable	Percentage %	Reference
HR+/HER2- population	78.8	MNCR[2]
Early breast cancer proportion	52.1	CPG Management of Breast Cancer[6]
New breast cancer cases/year (2020)	8,418	Globocan 2020

6.1.3 Model Input

a. Effectiveness Data

The outcome proportions in this study were obtained from published clinical trials as shown in Table 19. The main outcomes from these clinical trials were the proportion of population according to risk of recurrence, who had received chemotherapy guided by the molecular profiling assay Oncotype DX and MammaPrint.

Table 19. Outcome data

Variable	Risk	Proportion receiving chemotherapy (%)	Reference
OncotypeDX guided	Low	21.2	Vallireal-Garza et al,2020[20]
	Intermediate	44.1	
	High	91.7	
MammaPrint guided	Low	19.0	IMPACt trial. Soliman H. et al, 2020[40]
	High	83.5	

b. Resources and Cost Data

The costs used in this analysis were based on MOH Casemix Data[5] price per case data, and personal communication with the expert committee, private hospitals, oncology pharmacists from MOH Hospitals. Direct medical costs included were cost of drugs, cost of systemic therapy, cost of molecular profiling assay, cost of complication related management, and cost of specialist clinic follow-ups. All costs are expressed in Malaysian Ringgit (MYR) for year 2021. All results were presented as cost and cost savings, and overall savings per year.

6.1.4 Model Assumptions

The following key assumptions were used in this model:

1. The usual care using the non-genetic prognostic tools (St Gallens, PREDICT online tool, Adjuvant! Online).
2. Current practice of non-genetic risk profiling tools guide management to avoid chemotherapy in low risk patients, and intermediate and high risk patients will receive chemotherapy
3. There is no multiple testing. Each person tested receives a single molecular profiling test.
4. The population does not take into account of isolated ER+, HER2- men diagnosed with early-stage breast cancer.
5. All patients recommended chemotherapy will be compliant to recommendation of molecular profiling assay score-concordant treatment

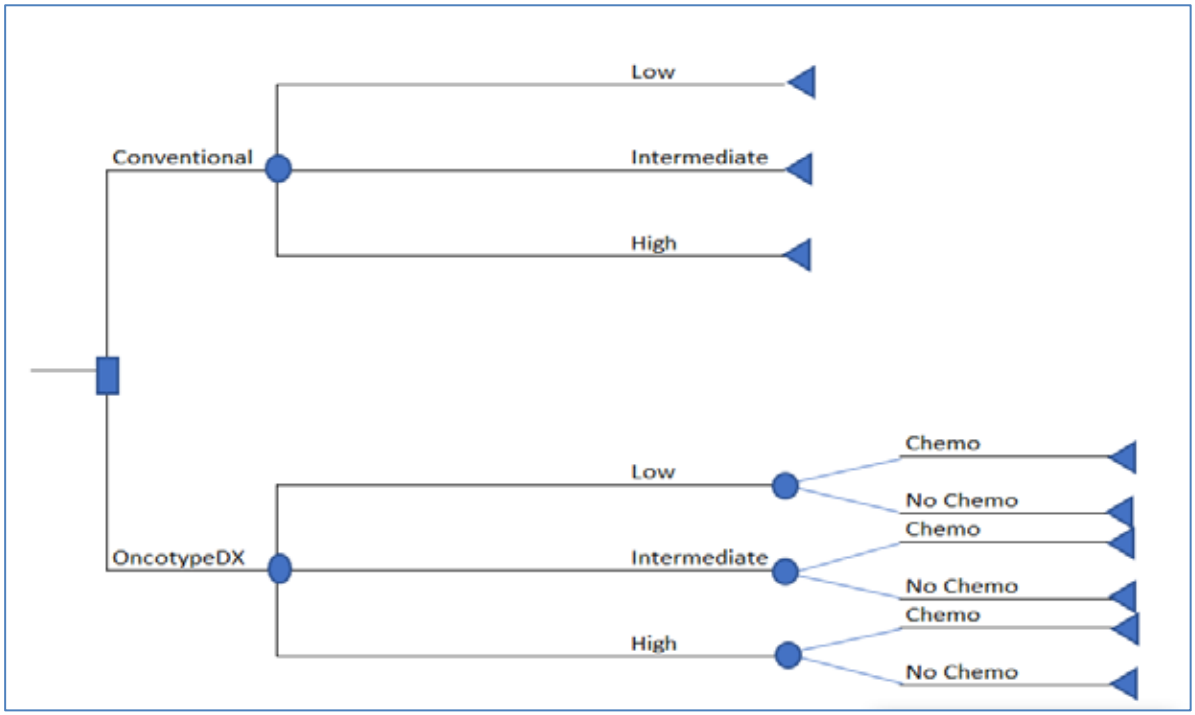


Figure 10: Decision Tree Model for HR+ HER2- Patient Using Oncotype DX

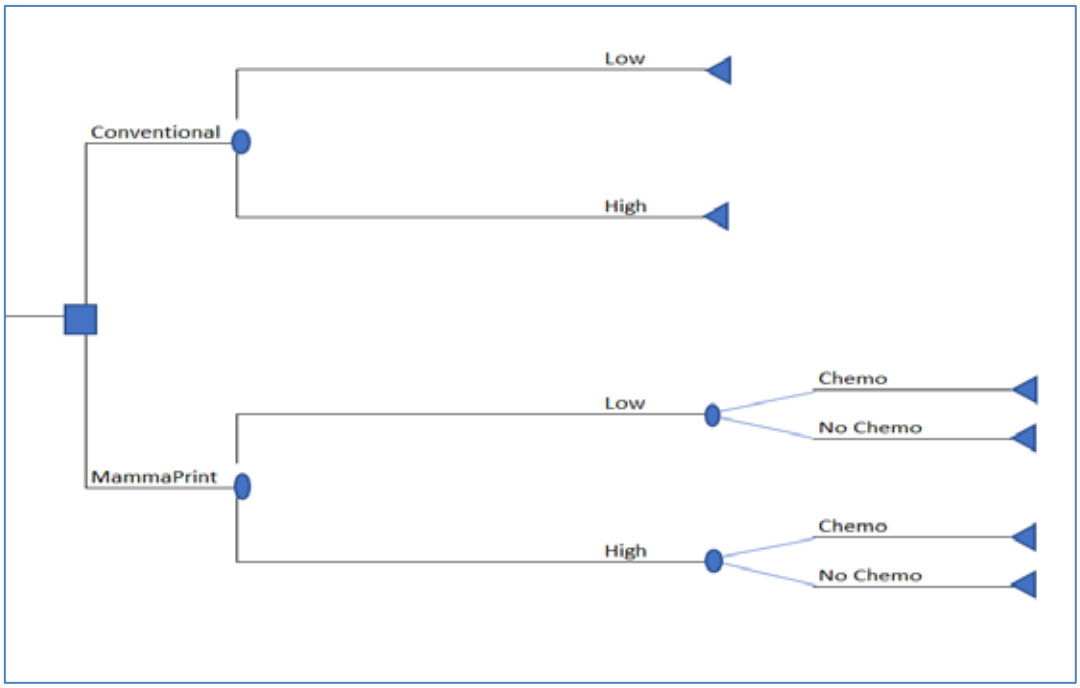


Figure 11: Decision Tree Model for HR+ HER2- Patient using MammaPrint

6.2 RESULTS & DISCUSSION

The main outcome of the decision-analytic model is cost savings, and overall incremental cost per year for the number of patients that would benefit from chemotherapy averted.

The results of this model reflected the cost saved from aversion of chemotherapy if molecular profiling assays (Oncotype DX or MammaPrint) are used compared to conventional non-genetic risk prognostic tools in guiding chemotherapy initiation for high-risk early HR-positive HER2-negative breast cancer patients, according to lymph node involvement. Cost analysis of the evaluated strategies were presented in Table 20 – 21.

As presented in Table 20a, the use of risk stratified Oncotype DX guided chemotherapy compared to conventional method in low and high risk of recurrence group of LN-negative patient cohorts gave an incremental cost of MYR 23,895,713.28 and MYR 4,149,485.04 per year, respectively. However, its use in the intermediate risk of recurrence group of LN-negative patient's cohort showed cost savings of MYR 10,703,458.56. With these, the overall incremental cost per year of using risk stratified Oncotype DX guided chemotherapy versus conventional method was MYR 17,341,739.76. There were 647 LN-negative patients who averted chemotherapy and the complications, where 616 of them were from the intermediate risk of recurrence group.

Simulated in LN-positive positive patients, the use of risk stratified Oncotype DX guided chemotherapy gave an overall incremental cost per year of MYR 7,540,934.88. There was cost savings of MYR 4,447,623.36 in intermediate risk of recurrence, where there were 277 patients who averted chemotherapy and its complications, 264 of them from the intermediate risk of recurrence group. This is seen in Table 20b.

Oncotype DX guided chemotherapy gave an accrued incremental cost per year of MYR 24,882,674.64. In addition to the identification of patients who averted chemotherapy, out of the 1400 patients classified with low risk of recurrence using conventional method, 297 of them were identified for chemotherapy after using Oncotype DX (89 in the LN-negative group, and 208 in the LN-positive group).

Table 20. Cost of risk stratified Oncotype DX guided chemotherapy and savings in LN- and LN+

a) Lymph node negative patient cohort of 2,450 patients

Risk	Conventional cost (MYR)	No. of patients	Post guidance cost (MYR)	Cost savings (MYR)	Cost incurred (MYR)
Low	1,452,752.00	**980**	25,348,465.28		23,895,713.28
Intermediate	50,353,201.12	**1102**	39,649,742.56	10,703,458.56	
High	16,814,862.08	**368**	20,964,347.12		4,149,485.04
Overall incremental cost per year (MYR)					**17,341,739.76**

b) Lymph Node positive patient cohort of 1,050 patients

Risk	Conventional cost (MYR)	No. of patients	Post guidance cost (MYR)	Cost saving (MYR)	Cost incurred (MYR)
Low	9,683,074.80	**420**	19,869,281.16		10,186,206.36
Intermediate	31,491,924.96	**472**	27,044,301.60	4,447,623.36	
High	10,541,788.44	**158**	12,344,140.32		1,802,351.88
Overall incremental cost per year (MYR)					7,540,934.88

The use of risk stratified MammaPrint guided chemotherapy and cost incurred are shown in Table 21. Simulated in LN-negative 2450 patients, and 1050 LN-positive patients, there was an incremental cost of MYR 67,395,212.24 and MYR 28,869,914.40 respectively. A total of 217 patients averted chemotherapy, and 416 low risk of recurrence patients by conventional method were identified for chemotherapy with MammaPrint assay.

Table 21. Cost of risk stratified MammaPrint guided chemotherapy in LN- and LN+

a) Lymph node negative patient cohort of 2,450 patients

Risk	Conventional cost (MYR)	Number of patients	Post guidance cost (MYR)	Cost incurred (MYR)
Low	2,269,554.40	**1531**	53,409,710.96	51,140,156.56
High	41,991,462.64	**919**	58,246,518.32	16,255,055.68
Overall incremental cost per year (MYR)				**67,395,212.24**

b) Lymph node positive patient cohort of 1,050 patients

Risk	Conventional cost (MYR)	Number of patients	Post guidance cost (MYR)	Cost incurred (MYR)
Low	15,124,040.64	**656**	36,982,195.64	21,858,155.00
High	26,287,750.92	**394**	33,299,510.32	7,011,759.40
Overall incremental cost per year (MYR)				**28,869,914.40**

Cost savings of approximately MYR 27 million were seen with usage of Oncotype DX for chemotherapy guidance in early HR-positive HER2-negative breast cancer irregardless of LN status, with 924 patients who averted chemotherapy.

In contrast, for the cohort of 3,500 patients simulated, usage of MammaPrint incurred incremental cost of MYR 67,395,212.24 in LN-negative patients and MYR 28,869,914.40 in LN-positive patients. This resulted in an accrued incremental cost of MYR 96,265,126.64 if all eligible 3,500 were tested with MammaPrint.

Budget Implication

To achieve the maximal benefit of cost savings and aversion of chemotherapy with its complications, the budget incurred for investment in targeted testing in the intermediate risk group using Oncotype DX assay was calculated. Oncotype DX assay procurement for 1574 patients will incur MYR 23,610,000.

Sensitivity Analysis

Molecular profiling assay price (50%-60%)

The decision analytic model was simulated to reduce cost of the molecular profiling assay of 50% to 60% of the price, seen in Table 22-23.

Although it is still cost saving for the intermediate risk group for those that used the Oncotype DX assay, the overall cost is found to be cost saving to a total of MYR1,367,325.36 when the price is reduced to 50%, while still yielding an accrued incremental cost of MYR3,882,674.64 when the price is reduced to 60%. When there is 50% reduced cost, the cost savings yielded in the intermediate risk group is MYR 26,956,081.92. Whereas the cost savings yielded in the intermediate risk group when the assay price is reduced to 60%, is MYR 24, 595,081.92.

With the reduction of 50% of the Oncotype DX assay price, there is a potential savings of testing the whole cohort, regardless of LN and risk status.

The sensitivity analysis simulated in the 3,500-patient cohort using MammaPrint guided chemotherapy, did not show cost savings. It incurred an overall incremental cost of MYR 61,265,126.64 when there was a reduction to 60% of the assay price, and MYR 52,515,126.64 after reduction to 50% of the assay price.

If price negotiations of Oncotype DX can be done to procure the assay at reduction of 50% of the quoted price, the molecular profiling may benefit and potentially have greater access to the eligible patient population regardless of LN and risk status. The budget required for procurement of 3,500 Oncotype DX assay is MYR 26,250,000.00.

This economic evaluation was done as cost benefit analysis of a short-term outcome, namely chemotherapy guidance and chemotherapy averted. This is because concordance analyses do not report long-term outcome. (Concordance is defined as the degree to which tests assign

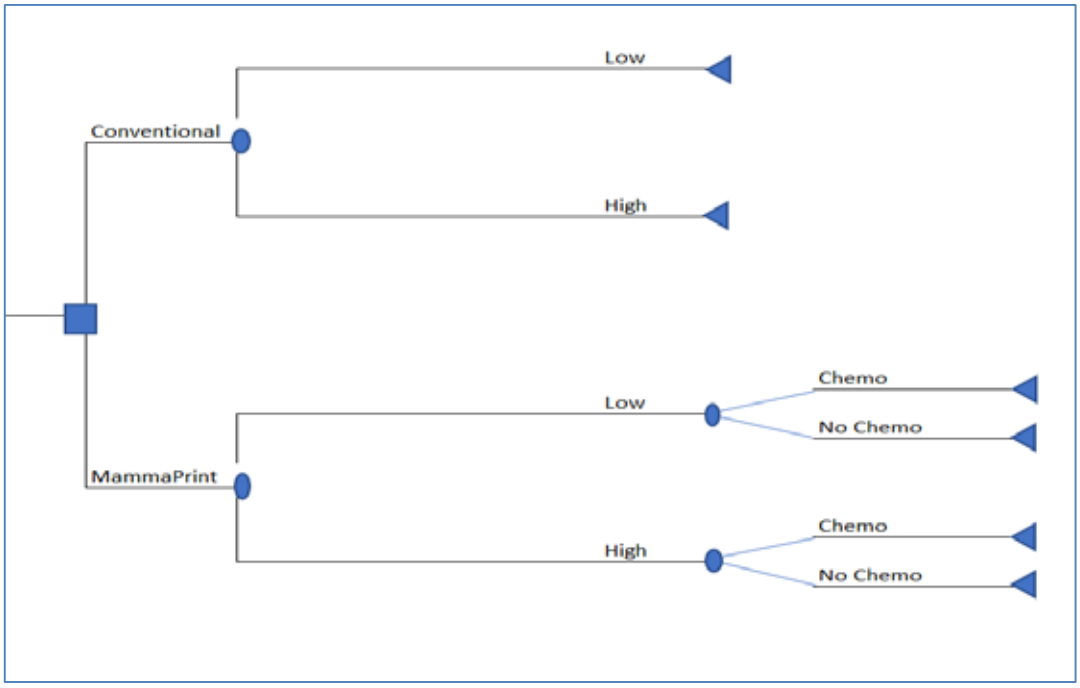


Figure 11: Decision Tree Model for HR+ HER2- Patient using MammaPrint

6.2 RESULTS & DISCUSSION

The main outcome of the decision-analytic model is cost savings, and overall incremental cost per year for the number of patients that would benefit from chemotherapy averted.

The results of this model reflected the cost saved from aversion of chemotherapy if molecular profiling assays (Oncotype DX or MammaPrint) are used compared to conventional non-genetic risk prognostic tools in guiding chemotherapy initiation for high-risk early HR-positive HER2-negative breast cancer patients, according to lymph node involvement. Cost analysis of the evaluated strategies were presented in Table 20 – 21.

As presented in Table 20a, the use of risk stratified Oncotype DX guided chemotherapy compared to conventional method in low and high risk of recurrence group of LN-negative patient cohorts gave an incremental cost of MYR 23,895,713.28 and MYR 4,149,485.04 per year, respectively. However, its use in the intermediate risk of recurrence group of LN-negative patient's cohort showed cost savings of MYR 10,703,458.56. With these, the overall incremental cost per year of using risk stratified Oncotype DX guided chemotherapy versus conventional method was MYR 17,341,739.76. There were 647 LN-negative patients who

Table 22. Sensitivity analysis with reduction of cost of assay (50-60%) of risk stratified Oncotype DX guided chemotherapy and savings in LN- and LN+

a) Lymph node negative patient cohort of 2,450 patients

Risk	Conventional cost (MYR)	Post guidance cost, Assay @ 50% (MYR)	Incremental cost (MYR)	Post guidance cost, Assay@ 60% (MYR)	Incremental cost (MYR)
Low	1,452,752.00	**17,998,465.28**	16,545,713.28	**19,468,465.28**	**18,015,713.28**
Intermediate	50,353,201.12	**31,384,742.56**	-18,968,458.56	33,037,742.56	-17,315,458.56
High	16,814,862.08	**18,204,347.12**	1,389,485.04	**18,756,347.12**	**1,941,485.04**
Overall incremental cost per year (MYR)			**-1,033,260.24**		**2,641,739.76**

Legend: Green box, savings.

b) Lymph node positive patient cohort of 1,050 patients

Risk	Conventional cost (MYR)	Post guidance cost, Assay @ 50% (MYR)	Cost incremental	Post guidance cost, Assay@ 60% (MYR)	Cost incremental (MYR)
Low	1,452,752.00	**16,719,281.16**	7,036.206.36	**17,349,281.16**	**7,666,206.36**
Intermediate	50,353,201.12	**23,504,301.60**	-7,987,623.36	24,212,301.60	-7,279,623.36
High	16,814,862.08	**11,159,140.32**	617,351.88	**11,396,140.32**	**854,351.88**
Overall incremental cost per year (MYR)			**-334,065.12**		**1,240,934.88**

Legend: Green box, savings.

Table 23. Sensitivity analysis with reduction of cost of assay (50-60%) of risk stratified MammaPrint guided chemotherapy

a) Lymph node negative patient cohort of 2,450 patients

Risk	Conventional cost (MYR)	Post guidance cost, Assay @ 50% (MYR)	Incremental cost (MYR)	Post guidance cost, Assay@ 60% (MYR)	Incremental cost (MYR)
Low	2,269,554.40	34,272,210.96	32,002,656.56	38,099,710.96	35,830,156.56
High	41,991,462.64	46,759,018.32	4,767,555.68	49,056,518.32	7,065,055.68
Overall incremental cost per year (MYR)			36,7703,212.24		**42,895,212.24**

b) Lymph node positive patient cohort of 1,050 patients

Risk	Conventional cost (MYR)	Post guidance cost, Assay @ 50% (MYR)	Incremental cost (MYR)	Post guidance cost, Assay@ 60% (MYR)	Incremental cost (MYR)
Low	15,124,040.64	28,782,195.64	13,658,155.00	30,422,195.64	15,298,155.00
High	26,287,750.92	28,374,510.32	2,086,759.40	29,359,510.32	3,071,759.40
Overall incremental cost per year (MYR)			15,744,914.40		**18,369,914.40**

There are a few barriers to embracing the use of predictive value of the molecular profiling assays for chemotherapy benefits fully. Ithimakin S et al noted from a survey that reimbursement of the molecular profiling assays is a challenge in six other Asian countries, namely Japan, South Korea, Singapore, Thailand, Hong Kong SAR, Taiwan. As of November 2021, three commercial tests Oncotype DX, MammaPrint and Prosigna were not reimbursed. This was in spite the countries surveyed were high income countries and upper middle-income countries. All four tests (Oncotype DX, MammaPrint, Prosigna, and EndoPredict) required the samples were to be sent for processing outside each territory.[42]

Jeyasekera and Mandeblatt reviewed that Oncotype DX was the most assessed gene expression profile testing, and many reported cost savings or cost-effective results when Oncotype DX was incorporated with clinical features to guide treatment decisions in patient subgroups.[43] This was similar to the finding of our decision analytic model that Oncotype DX yielded cost saving impact for the intermediate risk subgroup.

Moving forward, it is good to have appropriate genomic tests available, as the American Joint Committee on Cancer (AJCC) Staging Manual 8th edition requires inclusion of prognostic factors since 2018.[44] They included the use of Oncotype DX, MammaPrint, EndoPredict, PAM 50 Prosigna and Breast cancer index as genomic tests to be included to aid staging.[45]

However, it is to be noted that use of molecular profiling assays may present delay in chemotherapy initiation,[45] when 31% out of 263 US patients with Oncotype DX test ordered had a delay of ≥42 days from surgery to chemotherapy initiation, compared to 20% for other patients,[46] and there was a median handling time of 3 working days[47] without considering the outsourcing method we would have to employ in our region.

7.0 CONCLUSION

Molecular profiling assays are significantly effective in prognosticating between low-risk and high-risk of recurrence among patients with HR+/HER2-ve early-stage breast cancer. However, further assessment is required in terms of predicting of chemotherapy benefit, Oncotype DX and MammaPrint are able to predict the chemotherapy benefit regardless of lymph-nodes status. Individual prospective assays are available but there are not head to head prospective study to compare between the assays. Retrospective study looking at the association and correlations between the assays are limited in number and has small sample size (<100). Each assay had poor to weak association with each other and should not be used interchangeably. Overall, LN- and low-risk early breast cancer patients might benefit more from molecular profiling assays. Economically wise, the molecular profiling assays were cost-effective compared to conventional method and Oncotype DX was the most commonly used.

In economic evaluation, both Oncotype DX and MammaPrint incurred incremental cost if utilized for testing the whole eligible population. However, cost savings of approximately MYR 15,151,081.92 can be seen with usage of Oncotype DX in both intermediate risk of recurrence LN-negative and LN-positive breast cancer patients with 880 patients who averted chemotherapy. Therefore, maximal cost savings and potential benefits in averted chemotherapy with its complications may be achieved if targeted testing was performed using Oncotype DX in the intermediate risk of recurrence group. The budget implications to procure Oncotype DX assays for 1574 patients would be MYR 23,610,000.00.

The sensitivity analysis showed that overall cost savings can be achieved if the price of Oncotype DX is reduced to 50% of the quoted price, giving a total accrued cost savings of MYR 1,367,325.36. If price negotiation can be done, a minimum reduction of 50% of the Oncotype DX price may potentially offer eligible population greater access to Oncotype DX assay regardless of LN status or risk. The budget required for procurement of Oncotype DX assay for 3,500 patients with reduction to 50% of the quoted price is MYR 26,250,000.00.

8.0 RECOMMENDATION

Molecular profiling assays has a role in discriminating recurrence risk in HR+/HER2- early-stage breast cancer patients. Oncotype DX may be recommended in management of HR+/HER2- early breast cancer with the maximal potential benefit in the intermediate risk of recurrence group with purchasing price negotiation.

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APPENDIX 1: HIERARCHY OF EVIDENCE FOR EFFECTIVENESS STUDIES

DESIGNATION OF LEVELS OF EVIDENCE

I	Evidence obtained from at least one properly designed randomised controlled trial.
II-I	Evidence obtained from well-designed controlled trials without randomisation.
II-2	Evidence obtained from well-designed cohort or case-control analytic studies, preferably from more than one centre or research group.
II-3	Evidence obtained from multiple time series with or without the intervention. Dramatic results in uncontrolled experiments (such as the results of the introduction of penicillin treatment in the 1940s) could also be regarded as this type of evidence.
III	Opinions or respected authorities, based on clinical experience; descriptive studies and case reports; or reports of expert committees.

SOURCE: US/CANADIAN PREVENTIVE SERVICES TASK FORCE (Harris 2001)

APPENDIX 2: HEALTH TECHNOLOGY ASSESSMENT PROTOCOL

MOLECULAR PROFILING IN BREAST CANCER

1.0 BACKGROUND INFORMATION

The most recent Malaysian National Cancer Registry (MNCR) 2012-2016 showed an increasing trend of breast cancer cases from 18,206 (MNCR 2007 – 2011) report to 21,634 (current MNCR 2012 – 2016).[1,2] According to Globocan 2020, 17.3% (8,418) of new breast cancer cases was reported in Malaysia in 2020.[3] Approximately 48% of breast cancer cases in Malaysia are diagnosed late with age standardised incidence rate (ASR) is 34.1 per 100,000 populations.[4] Thus, as an alternative of encouraging women to examine their breast, the government provides subsidised mammograms through the Ministry of Women and Family Development (LPPKN) and state government programmes. Unfortunately, the level of breast cancer screening utilisation in Malaysia is low probably influenced by educational level, socioeconomic status, cultural perception and beliefs of women and community.[1]

There are several risk factors of breast cancer that can be divided into non-modifiable and modifiable. The one that non-modified and currently play an important role in treatment choice is genetic factor or genetic mutation.[5] According to Clinical Practice Guideline (CPG) Management of Breast Cancer (3rd Edition), there are advancements in screening method, early prognosis even in treatment modalities over the years. This included the one that involving molecular subtyping that getting essentials and even become an important factor in treatment response. Advances in molecular biology and pharmacology aids in better understanding of breast cancer, enabling the design of effective therapy to target the cancer and responds efficiently.[6]

In general, molecular profiling is a scientific approach that compare different types of tissues at a molecular level (DNA, mRNA or protein) on a global scale.[7] The molecular profiling test is more on genomic technology especially in predicting individual patient's prognosis by interpreting the expression pattern of a panel of specific tumour-related genes.[8] The genomic test looks at all the genes and examine how the genes interact and affect health.[9] The transcription of specific set of genes is used as a surrogate marker for metastatic potential. The gene expression pattern and specific gene expression threshold levels can identify the tumours with more aggressive biology, thereby quantifying the risk of recurrence more accurately with more aggressive treatment.[8] As for genetic testing, it is designed to detect a single gene mutation associated with specific cancer such as BRCA1 and BRCA2 mutations that associated with breast and ovarian cancer.[9]

Three subtypes of breast tumours with different biologic behaviours were discovered using the traditional ImmunoHistoChemistry (IHC) techniques: hormone-receptor-positive, triple negative, and Human Epidermal Receptor (HER) 2/neu-positive breast cancers. All of these

subtypes have distinct natural histories, which require different management approaches and the availability of expression profiling and hierarchical clustering enabled to identify the additional subtypes. Breast cancer comprises of at least 7 different biologic subtypes. They include luminal A, luminal B, luminal C, HER2-enriched, basal-like, claudin-low, and normal breast-like.[8] As an example, patients who are identified with early-oestrogen receptor-positive (ER) lymph node negative (LN-) breast cancer are likely to have higher risk of recurrence. Meanwhile, patients who are identified as low risk may be avoiding possible unnecessary treatment as well as the short or long-term side effects that associated with chemotherapy.[5]

Thus, the molecular profiling tests aim to improve the use of chemotherapy in breast cancer by improving the categorisation of patients in accordance with risk and the identification of those patients who will gain most benefit from chemotherapy.[10] There are several commercially available molecular profiling tests including Oncotype DX, Prosigna (PAM 50), EndoPredict and MammaPrint. The tests are typically performed after surgery once hormone and lymph node status are known including other information such as tumour size and grade.[11] All four molecular profiling tests already approved by Food and Drug Administration (FDA) agency and European Medicines Agency (EMA) and their information are summarised in the Table 1.

Table 1: Molecular profiling test used for chemotherapy decision-making in ER-positive, ERBB2 (HER2)-negative breast cancer

Informations	MammaPrint	Oncotype DX	Prosigna (PAM50)	EndoPredict
Number of genes	70	21	50	11
Method	DNA microarray	RT-PCR	Nanostring	RT-PCR
Tissue sample type	Frozen/FFPE	FFPE	FFPE	FFPE
Location	Central	Central	Local	Local
Test results	High or low risk +subtype	High, Intermediate or low risk	High, intermediate or low risk +subtype	High or low risk
Clinical Indication (according to EGTM)	Predicting prognosis and guiding decision-making regarding chemotherapy for women with ER+/HER2- EBC, LN- or LN+ (1-3)	Predicting prognosis and guiding decision-making regarding chemotherapy for women with ER+/HER2- EBC, LN- or LN+ (1-3)	Predicting prognosis and guiding decision making regarding chemotherapy for women with ER+/HER2- EBC, LN- or LN+ (1-3)	Predicting prognosis and guiding decision-making regarding chemotherapy for women with ER+/HER2- EBC, LN- or LN+ (1-3)
Prospective validation trial(s)	MINDACT (positive)	TAILORx (positive) and RxPONDER (ongoing)	OPTIMA (ongoing)	None
Regulatory approval	EMA, FDA	EMA, FDA	EMA, FDA	EMA, FDA

Informations	MammaPrint	Oncotype DX	Prosigna (PAM50)	EndoPredict
Original validation set	Developed in young patients (aged <55 years) who had not received therapy after surgery	Developed in patients who had received tamoxifen only in the NSABP B-20 and B-14 trials	Postmenopausal patients in the training and development sets received heterogeneous treatment	Developed in postmenopausal patients who had received endocrine therapy only in the ABCSG-6 and -8 trials

*Table adapted from WHO BlueBooks[12]
FFPE: Formalin-fixed, paraffin-embedded; EGTM: European Group on Tumour Markers

a)Oncotype Dx

b)MammaPrint

c)Prosigna

d)EndoPredict

Figure 1: Various Molecular Profiling Tests

a) https://www.breastcancer-news.com
b) https://www.medgadget.com/2008/12/mammaprint_identifies_low_risk_her2_patients.html
c) https://www.businesswire.com/news/home/20140805006562/en/NanoString-Technologies-Receives-Market-Approval-From-the-Australian-Therapeutic-Goods-Administration-for-Its-Prosigna-Breast-Cancer-Prognostic-Gene-Signature-Assay
d) https://www.sciencewerke.com/all_products/myriad-endopredict/

Reasons for request

More demands are coming from patients and clinicians to use gene assays profiling as part of management of breast cancer. However, the in-depth knowledge of types available, usefulness, cost-effectiveness is not readily available for clinicians to make a sound decision on these assays.

2.0 POLICY QUESTION

2.1 Does molecular profiling as part of breast cancer management beneficial to predict the recurrence risk?

2.2 Should molecular profiling be part of breast cancer management in Ministry of Health (MOH)?

3.0 OBJECTIVES

3.1 To assess the relative effectiveness and safety of different types of molecular profiling and subsequent management in breast cancer. *(As results of this, decision to give or not to give chemotherapy will determine patient outcomes such as mortality, and quality of life [QoL])*.

3.2 To assess the economic implication, social, ethical, and organisational aspects related to molecular profiling of breast cancer.

The following **research questions** will be addressed:

3.1.1 What is the accuracy/ performance of different types of molecular profiling in predicting recurrence risk?

3.1.2 Is molecular profiling cost-effective?

3.1.3 Which is the best molecular profiling in terms of accuracy and cost-effective?

3.1.4 What is the social, ethical, and organisational implication/ impact related to molecular profiling?

3.1.5 Which population can benefit the molecular profiling?

4.0 METHODS

4.1 Search Strategy

Electronic database will be searched for published literatures pertaining to molecular profiling for early breast cancer detection.

4.1.1 Databases as follows: MEDLINE, EMBASE, PubMed, EBM Reviews-Cochrane Database of Systematic Review, EBM-Reviews-Cochrane Central Register of Controlled Trials, EBM Reviews-Health Technology Assessment, EBM Reviews-NHS Economic Evaluation Database, Database of Abstracts of Reviews of Effects (DARE), INAHTA Database, HTA database and FDA database.

4.1.2 Additional literatures will be identified from the references of the retrieved articles.

4.1.3 General search engine will be used to get additional web-based information if there is no retrievable evidence from the scientific databases.

4.1.4 There will be no limitation applied in the search such as year and language.

4.1.5 The search strategy will be included in the appendix.

4.2 Inclusion and Exclusion Criteria

4.2.1 Inclusion criteria

a. Population : Early-stage breast cancer lymph node status *(LN-positive [LN+, n0, n1], LN-negative [LN-])*, and receptor status *(ER-positive [ER+], HER2-negative [HER2-])* and pre- and post-menopausal women

b. Intervention : Molecular profiling / gene expression profiling (GEP) / tumour profiling test (Oncotype DX, MammaPrint, EndoPredict, Prosigna and immunochemistry 4 (IHC4))

c. Comparators : i.Comparing among molecular profiling tests
i.No comparator

d. Outcome : i.Effectiveness: Prognostic performance *(Recurrence Score [RS], Risk of Recurrence [ROS] score)*, prediction of systemic treatment benefit, breast cancer-related mortality, quality of life (QoL)
i.Safety: adverse events, complications
i.Economic implications: cost-effectiveness, cost-utility, cost-benefit analysis

v. Potential psychological and behavioural harms and benefits of test results
vi. Training requirements or learning curve

e. Study design : HTA reports, systematic reviews (SRs) with/out meta-analysis (MA) / network MA, randomised controlled trials (RCTs), cohort studies, and economic evaluation

f. English full text articles

4.2.2 Exclusion criteria

a. Study design : Animal study, laboratory study, case-control, case report, case series, narrative review

b. Non-English full text articles

Based on the above inclusion and exclusion criteria, study selection will be carried out independently by two reviewers. Disagreement will be resolved by discussion.

4.3 Critical Appraisal of Literature

The risk of bias of all retrieved literatures will be assessed using the relevant checklist of Critical Appraisal Skill Programme (CASP) and Cochrane risk of bias tool for randomised trials (RoB 2).

4.4 Analysis and Synthesis of Evidence

4.4.1 Data extraction strategy

The following data will be extracted:

i. Details of methods and study population characteristics
ii. Detail of intervention and comparators
iii. Details of individual outcomes specified

Data will be extracted from selected studies by a reviewer using a pre-designed data extraction form and checked by another reviewer. Disagreements will be resolved by discussion.

4.4.2 Methods of data synthesis

Data on the accuracy, safety and cost-effectiveness associated with molecular profiling in breast cancer will be presented in tabulated format with narrative summaries. Meta-analysis may be conducted for this HTA.

5.0 REPORT WRITING

6.0 REFERENCES

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APPENDIX 3: SEARCH STRATEGY

Database: Ovid MEDLINE(R) ALL <1946 to October 27, 2022>
Search Strategy:

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1. Breast Neoplasms/
2. (breast adj1 (cancer or carcinoma* or neoplasm*)).tw.
3. (breast adj1 tumor*).tw
4. (mammary adj1 cancer*).tw
5. (breast malignant adj2 (neoplasm* or tumor*)).tw.
6. (human mammary adj2 (carcinoma* or neoplasm*)).tw.
7. (cancer adj3 breast).tw
8. (malignant neoplasm adj3 breast).tw
9. Gene Expression Profiling/
10. (gene expression adj2 (monitoring* or profiling* or pattern analysis)).tw
11. transcript expression analys*.tw
12. mrna differential display*.tw
13. (transcriptome adj1 (analys* or profiling*)).tw
14. Molecular profiling.tw
15. Gene Expression Regulation/
16. (gene adj2 (action regulation or expression regulation)).tw
17. (regulation adj3 gene expression).tw
18. gene expres$ assay.tw
19. Genomics/ (61479)
20. genomic*.tw
21. (comparative adj1 genomic*).tw
22. Genomic test.tw
23. Biomarkers, Tumor/
24. ((biochemical or biologic*) adj2 tumor marker*).tw
25. ((tumor or neoplasm) adj2 metabolite marker*).tw
26. ((cancer or tumor) adj1 biomarker*).tw
27. ((carcinogen or tumor) adj1 marker*).tw
28. Genomic profiling.tw
29. Biopsy/
30. biops$.tw
31. limit 39 to (humans and yr="2000 - Current")
32. limit 40 to (clinical study or clinical trial, all or comparative study or controlled clinical trial or government publication or meta-analysis or multicenter study or observational study or randomized controlled trial or "systematic review")
33. Prosigna.mp
34. Prosigna.tw
35. PAM50.mp
36. PAM50.tw
37. OncotypeDX.mp
38. Oncotype DX.mp
39. OncotypeDX.tw
40. Oncotype DX.tw
41. MammaPrint.mp
42. MammaPrint.tw)
43. EndoPredict.mp
44. EndoPredict.tw

APPENDIX 4: EVIDENCE TABLE

<Upon request>

APPENDIX 5: LIST OF EXCLUDED STUDIES

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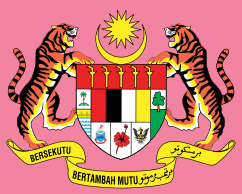

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